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(54) Title: MAMMALIAN gb2 $GABA_B$ RECEPTORS

(57) Abstract

The present invention provides a mammalian $GABA_B$ receptor subunit polypeptide, namely the gb2 subunit, as well as a DNA encoding the subunit. The gb2 subunit, when in conjunction with a mammalian gb1 polypeptide, expresses $GABA_B$ receptor activity. Vectors for expressing the gb2 polypeptide and cells expressing it are part of the invention, as are methods of producing a gb2 $GABA_B$ receptor subunit in a mammalian cell, determining the ability of a substance to bind to a mammalian $GABA_B$ receptor, and evaluating whether a substance modulates an expressed activity of a $GABA_B$ receptor. The invention also discloses an antibody that reacts immunospecifically with a mammalian gb2 receptor subunit.

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MAMMALIAN gb2 $GABA_B$ RECEPTORS

Field of The Invention

The present invention relates to a new subtype of mammalian $GABA_B$ receptor. This subtype has been designated gb2 $GABA_B$. In particular, the present invention relates to nucleic acids (DNA) encoding gb2 $GABA_B$, to vectors and host expression systems containing these nucleic acids, to isolated and recombinantly-produced antigens encoded by these nucleic acids, and to antibodies produced against these antigens.

Background

GABA (γ-aminobutyric acid or 4-aminobutanoic acid) is the major inhibitory neurotransmitter in the mammalian brain. GABAergic inhibitory synaptic transmission is mediated by two distinct types of receptors, ligand-gated chloride channels and G-protein-coupled receptors. The multi-subunit $GABA_A$ receptors constitute the ligand-gated channels that when activated allow the passage of chloride ions into the cell resulting in hyperpolarization and cessation of neurotransmission. $GABA_B$ receptors, which are abundantly and heterogeneously distributed throughout the central nervous system, are members of the G-protein-coupled receptor superfamily and mediate a variety of inhibitory cellular processes through pertussis toxin-sensitive G-proteins G_o and/or G_i. The G-proteins (i.e., guanine nucleotide binding proteins) allow functional coupling of the $GABA_B$ receptors to Ca^{++} and K^+ channels. Activation of $GABA_B$ receptors has been shown to reduce evoked neurotransmitter release through modulation of voltage-sensitive Ca^{++} and/or K^+ currents, hyperpolarize a postsynaptic cell through activation of K^+ currents, and regulate adenylyl cyclase activity (see, for example, review articles by Bowery, Annu. Rev. Pharmacol. Toxicol. 33, 109-147 (1993); Kerr et al., Pharmac. Ther. 67, 187-246 (1995); Misgeld et al., Prog. Neurobiol. 46, 423-462 (1995)). $GABA_B$ receptors were originally identified due to their insensitivity to the $GABA_A$ antagonist bicuculline and their sensitivity to the agonist baclofen (Bowery et al., Eur. J. Pharmacol. 71, 53-70 (1981); Hill et al., Nature 290, 149-152 (1981)).

Second messenger and electrophysiological studies of pre- and postsynaptic $GABA_B$ receptors suggest that there may be as many as four receptor subtypes (Gemignani et al., Mol. Pharmacol. 46, 558-562 (1994); Cunningham et al., Brain Res. 720, 220-224 (1996); Bonanno et al., Trends Pharmacol. Sci. 14, 259-261 (1993)). Presynaptic $GABA_B$ autoreceptors have been shown to modulate GABA release, while heteroreceptors modulate the release of several neurotransmitters and peptides including L-glutamate, somatostatin, substance P, and cholecystokinin (Huston et al., Neurosci. 68, 465-478 (1995); Pende et al., Brain Res. 604, 325-330 (1993); Bonanno et al., Br. J. Pharmacol. 118, 1441-1446 (1996); Teoh et al., Br. J. Pharmacol. 118, 1153-1160 (1996); Raiteri et al., J. Pharmacol. Exp. Therapeut. 278, 747-751 (1996)). While the study of $GABA_A$ receptor structure and function has been advanced greatly by knowledge of their primary sequences, studies of the $GABA_B$ receptor have only recently begun. Kaupmann et al. (Nature 386, 239-246 (1997)) have described a sequence for a $GABA_B$ receptor which was isolated by expression cloning techniques. The identified receptors, rgb1a and rg1b, are members of the G protein-coupled seven transmembrane domain receptor family and are splice variants of the same receptor. These receptors exhibit the expected sensitivity and rank order of potency of $GABA_B$ receptor compounds that has been described in the literature for $GABA_B$ receptors in tissue preparations.

Summary of the Invention

The present invention relates to a new subtype of mammalian $GABA_B$ receptor which has been designated gb2 $GABA_B$. In particular, the present invention relates to nucleic acids (DNA) encoding gb2 $GABA_B$, to vectors and host expression systems containing these nucleic acids, to isolated and purified gb2 $GABA_B$ receptors, to isolated and recombinantly-produced antigens encoded by these nucleic acids, and to antibodies produced against these antigens. Using expressed sequence tag (EST) data base searches, polymerase chain reaction, and library screening, we have identified and isolated human and rat cDNAs for a novel $GABA_B$ receptors, designated hgb2 and rgb2, with homology to rgb 1a and rgb 1b (the two alternatively spliced forms of gb1).

This is only the second $GABA_B$ receptor primary sequence to be identified. The homology between rat receptor sequences (rgb1 and rgb2) and human receptor sequence (hgb2) indicates that they might be members of a larger $GABA_B$ receptor family. These receptors (i.e., hgb2, rgb2, and rgb1) comprise a new subfamily of seven transmembrane G-protein-coupled receptors that share structure and sequence similarities with the metabotropic glutamate receptors. Baclofen and GABA modulate cAMP synthesis through the gb2 receptors at μM concentrations. The rgb2 mRNA exhibits a more discrete expression pattern in rat brain than rgb1 and appears to be absent from white matter suggesting that it is predominantly localized to neurons.

One object of the invention is to provide a nucleic acid sequence that encodes a protein which comprises a mammalian gb2 $GABA_B$ receptor. A further object of the invention is to provide a nucleic acid sequence that encodes a protein which comprises a human gb2 $GABA_B$ receptor.

A further object of the invention is to provide constructs containing the genes encoding such receptors under the transcriptional and translational regulatory control of regulatory genes recognized by a desired host to which the mammalian gb2 $GABA_B$ receptor genes are foreign.

A still further object of the invention is to provide an isolated or pure clone from a mammalian tissue DNA library, wherein the clone contains a DNA sequence that encodes a mammalian gb2 $GABA_B$ receptor. Another object of the invention is to provide an isolated or pure clone from a human tissue DNA library, wherein the clone contains a DNA sequence that encodes a human gb2 $GABA_B$ receptor.

Another object of the invention is to provide an expression vector that contains the DNA of the invention and is capable of transforming a host cell.

Still another object of the invention is to provide host cells and have been transformed with such DNA and which express a mammalian gb2 $GABA_B$ receptor.

Another object of the invention is to provide isolated or purified proteins comprising mammalian gb2 $GABA_B$ receptors. Still another object of the invention is to provide an isolated or purified protein comprising human gb2 $GABA_B$ receptor.

Yet another object of the invention is to provide methods for obtaining a DNA sequence encoding a mammalian gb2 $GABA_B$ receptor, methods for preparing a cell

that expresses the receptor, and methods for using such cells in the pharmacological, physiological, functional, or other investigational analysis of GABA, $GABA_B$ receptors, and agonists or antagonists for $GABA_B$ receptors.

To achieve the objects and in accordance with the purpose of the invention, as embodied and broadly described herein, isolated or essentially pure DNA sequences encoding mammalian gb2 $GABA_B$ receptors are disclosed herein. Preferably, the DNA sequences are cDNA sequences, and most preferably they are the cDNA sequences shown in SEQ. ID NO.: 1 (human) and SEQ. ID NO.: 2 (rat) or allelic variants thereof. Preferably the mammalian gb2 $GABA_B$ receptors are hgb2 $GABA_B$ receptors as defined by SEQ. ID NO.: 3 (human) and rgb2 $GABA_B$ receptors as defined by SEQ. ID NO.: 4 (rat)) or allelic variants thereof.

Other objects and advantages of the present invention will be apparent from a consideration of the specification.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides schematic diagrams of hgb2 (panel A), rgb2 (panel B), rgb1b (panel C), and rgb1a (panel D) sequences and shows the relative location of oligonucleotide primers and restriction enzyme sites used in constructing the cDNAs and the location of oligonucleotide primers used to make riboprobes for *in situ* hybridization histochemistry. Left facing arrows represent sense primers, right facing arrows represent antisense primers. The cloning of hgb2 is detailed in Example 1; the cloning of rgb2 is detailed in Example 2; the cloning of rgb 1b and rgb 1a is detailed in Example 3.

Figure 2 illustrates the alignment of human gb2 and rat gb2 with rat gb1a and gb1b receptor amino acid sequences. Dots indicate residues identical in all receptors. Hydrophobic domains are denoted by bars and labeled with the transmembrane domain (TM) numbers. Potential N-linked glycosylation sites are designated by an asterisk over the appropriate residues.

Figure 3 illustrates hgb2 (panel A) and rgb1a (panel B) receptor-mediated modulation of forskolin-stimulated cAMP synthesis in hgb2/293-10 and rgb1a/293-18 cells. hgb2/293-10 and rgb1a/293-18 cells stably expressing the hgb2 and rgb1a receptors, respectively, were generated and assayed for modulation of forskolin-

stimulated cAMP synthesis as described in Example 8. Data are presented as the percent of total cAMP synthesized in the presence of forskolin only. Error bars represent the standard error of the mean for quadruplicate samples. The data presented are from a single representative experiment that has been replicated twice.

Figure 4 illustrates hgb2 mRNA expression in CNS and peripheral tissues as determined by Northern blot analysis. Clontech multiple human tissue Northerns were hybridized with a probe directed against the hgb2 receptor. Weak bands were observed in heart and spinal cord human mRNA.

Figure 5 illustrates the localization of rgb2 and rgb1 receptor mRNA by *in situ* hybridization histochemistry. Coronal 12 µm thin sections of a rat forebrain are shown. Brightfield (**A** and **C**) and darkfield (**B** and **D**) illumination is demonstrated of the identical visual fields; **A** and **B** show rgb1 and **C** and **D** show the rgb2 receptor mRNA. Note the lack of grains over the caudate nucleus and the septum in **C** and **D**. Abbreviations: c = caudate nucleus; cx = cerebral cortex; PO = preoptic area; s = septum. The scale bar represents 1 mm.

Figure 6 illustrates the localization of rgb2 and rgb1 receptor mRNA by *in situ* hybridization histochemistry. Coronal 12 µm thin sections of a rat forebrain are shown. Brightfield (**A** and **C**) and darkfield (**B** and **D**) illumination is demonstrated of the identical visual fields; **A** and **B** show the rgb1 and **C** and **D** show the rgb2 receptor mRNA. Note the lack of grains over the hypothalamus in **C** and **D**. Abbreviations: Hi = hippocampus; Hth = hypothalamus; Th = thalamus. The scale bar represents 1 mm.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to a new subtype of mammalian $GABA_B$ receptor which has been designated gb2 $GABA_B$. In particular, the present invention relates to nucleic acids (DNA) encoding gb2 $GABA_B$ receptors, to vectors and host expression systems containing these nucleic acids, to isolated and purified gb2 $GABA_B$ receptors, to isolated and recombinantly-produced antigens encoded by these nucleic acids, and to antibodies produced against these antigens. Using expressed sequence tag (EST) data base searches, polymerase chain reaction, and library screening, we have

identified and isolated human and rat cDNAs for novel mammalian $GABA_B$ receptors, designated hgb2 and rgb2, with homology to gb 1a and gb 1b.

The DNA of the invention is an isolated or essentially pure DNA sequence (i.e., polydeoxyribonucleotide) encoding a protein which comprises the new mammalian subtype gb2 $GABA_B$ receptors. As used herein, the term "isolated" and variations thereof means that the DNA is in isolation from DNA encoding proteins normally accompanying these receptors or encoding a different GABA receptor. Thus, the DNA of the invention includes DNA encoding the gb2 $GABA_B$ receptor when that DNA has been cloned into a bacterial vector, such as a plasmid, or into a viral vector that may be harbored by a bacteriophage, provided that such clones are isolated from clones that contain DNA encoding other proteins normally accompanying such receptors or encoding a different GABA receptor. As used herein, the term "essentially pure" and variants thereof means the DNA is substantially free of DNA and RNA that does not encode the gb2 $GABA_B$ receptor. That is, there will be no more than about 0.1 percent of other DNA and RNA and preferably no more than about 0.01 percent of other DNA and RNA in any sample that contains the DNA encoding the gb2 $GABA_B$ receptor.

Preferably, the DNA of the invention is complementary DNA (cDNA). One preferred human cDNA has the nucleotide sequence as defined in SEQ. ID No.:1; another preferred rat cDNA has the nucleotide sequence as defined in SEQ. ID No.:2. The cDNA is approximately 5.5 to 5.8 kilobases in length. The preferred human and rat cDNA code for proteins with the deduced amino acid sequence of SEQ. ID NO.:3 and SEQ. ID NO.:4, respectively. These proteins contain seven transmembrane regions (TM1 through TM7 as shown in Figure 2B). As those skilled in the art will realize, allelic variations of the DNA and protein which do not significantly affect the functionality of the DNA and/or protein can also been employed and considered within the invention described herein. For purposes of this invention, an "allelic variation" of a given DNA and/or protein retains at least about 95 percent of the sequence of the comparison molecule (DNA or protein) and maintains the essential functionality of the comparison molecule. For the DNA of this invention, the essential functionality is encoding a mammalian $GABA_B$ receptor of subtype gb2; for the protein of this invention, the essential functionality relates to its action as a

$GABA_B$ receptor. Thus, such allelic variations may involve single or multiple mutations so long as the functionality remains intact.

The DNA of the invention may be obtained by various methods, as detailed in the examples, involving known molecular biology techniques. The present invention also encompasses isolated or purified clones from mammalian tissue DNA library, which clone contains a DNA molecule encoding a mammalian gb2 $GABA_B$ receptor. Preferably, the clones comprise an essentially pure culture of bacteriophage containing the cDNA of SEQ. ID. NO.: 1 or SEQ. ID. NO.:2 inserted into the genome of the phage. Alternatively, the clone comprises an essentially pure culture of bacteria, such as *E. coli*, containing the cDNA of SEQ. ID. NO.: 1 or SEQ. ID. NO.:2 inserted into a plasmid (e.g., pUC-18, pBSSK II (-), pcDNA/amp, pcDNA3.1, and the like) in the bacteria. Cosmids, BACE YAC vectors, and λ phages could also be used as expression systems for these genes.

The DNA of the invention can be used to transform procaryotic cells, such as bacteria, fungi, or other microorganisms, or transfect eucaryotic cells, such as yeast or, mammalian cells. Transformation or transfection is accomplished by techniques known in the molecular biology. The DNA of the invention maybe joined to a wide variety of other DNA sequences for introduction into an appropriate host cell. The companion DNA will depend upon the nature of the host, the manner of introduction of the DNA into the host, and whether episomal maintenance or integration is desired. Mammalian cells are the preferred host.

Generally, DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognized by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed or transfected by the vector. Therefore, it will be necessary to select for transformed or transfected host cells. One selection technique involves incorporating into the expression vector a DNA sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance. Alter-

natively, the gene for such selectable trait can be on another vector, which is used to co-transform or co-transfect the desired host cell.

The preferred expression vector for use in the invention is the plasmid pcD3.1hgb2. This plasmid can be prepared from the expression vector pcDNA3.1.

After the modified expression vector has been prepared, it is transfected into a mammalian cell, using techniques known in the art. The mammalian cell may be any one that can be transfected with the DNA of the present invention.

Preferably, the cell is one that can be stably transfected with the DNA so that the DNA is integrated into the genome and expressed on an essentially continuous basis in the cell or its progeny. Further, the cell to be transfected is preferably one from a mammalian cell line that is capable of continuous growth in a suitable culture medium. Preferred types of cell lines include those derived from human embryonic kidney cells, such as HEK-293, neuronal cells, such as the type of pheochromocytoma cells known as PC 12 cells, fibroblast cells, such as A9 L cells, epithelial-like cells, such as Chinese hamster ovary (CHO) cells, and a glial or glial-like cells, such as c6 glioma cells.

After transfection, the cells are evaluated by screening to identify and isolate those that have taken up the expression vector. Preferably, the expression vector will be stably inserted into the genome of the cell. If the stably transfected cells were originally from a continuous cell line, the cell can be clonally expanded to provide a cell line that is capable of continuous growth in a suitable culture medium and which expresses a mammalian gb2 $GABA_B$ receptor.

A person skilled in the art, using known techniques and the teachings disclosed herein, can use the naturally variant or derived DNA of the invention to prepare sequencing vectors, expression vectors, transformed or transfected cells, and transfected cell lines as disclosed herein. Accordingly, these are within the scope of the present invention.

The cells and cell lines of the present invention can be used in the development of better drugs for the possible treatment of neurological and psychiatric disorders wherein $GABA_B$ receptors play an important role. The DNA, clones, and vectors of the present invention can be used for the construction of such cells and cell lines. Cells and cell lines that express mammalian gb2 $GABA_B$ receptors, particularly

those that express human gb2 $GABA_B$ receptors, will be extremely useful in the study of receptor function and its implications in neurological and psychiatric disorders. Relative to animal models, such cell culture assays are quick, inexpensive, and highly standardized. In addition, the cloned DNA sequences can be used to measure the mRNAs that encode these receptors, thus measuring tissue specific expression. Moreover, receptor pharmacology can be determined in isolation from other related receptors, including different GABA receptor types or subtypes. Therefore, these assays will be tools in the effort to provide a scientific basis for the rational development to new drugs.

In addition, the assays can be used to screen drugs in order to find or develop gb2 $GABA_B$ antagonists (substances that block the receptors) or agonists (substances that stimulate the receptors). Generally known techniques, modified in accordance with the discoveries and teachings disclosed herein, can be used with the cells and cell lines of the invention to evaluate potential antagonists or agonists by generating dose response, saturation, inhibition, or displacement curves. Initial candidates can be further tested for specificity, binding affinity, and activity.

Whether or not a chemical binds to a mammalian gb2 $GABA_B$ receptor can be determined by contacting the chemical with a cell or a part thereof from a host cell transformed by the DNA of the invention and determining the ability of the cell or part thereof to bind to the chemical in the appropriate assay in the presence of a chemical known to bind to a gb2 $GABA_B$ receptor, such as GABA and baclofen.

Gb2 $GABA_B$ receptor agonists are normally identified using both binding and functional assays. Such functional assays include the so-called second messenger assays. When neurotransmitters bind to receptors on the surface of cells, the metabolism of the various molecules is altered. When these molecules effect the physiology of the cell, they are referred to as second messengers. Two second messenger pathways that have been well characterized are inositol phosphate and cyclic adenosine monophosphate (cAMP) metabolism. See Berridge, Annu. Rev. Biochem. 56, 159 (1987) and Gilman, Annu. Rev. Bioahem. S6, 615 (1987). cAMP metabolism can be examined by various methods. The activity of the enzyme adenylate cyclase or the levels of cAMP can be measured. Thus, the invention provides an in vitro method for evaluating a chemical to determine if it is a gb2

$GABA_B$ receptor agonist. The chemical is contacted with a cell transformed by the DNA of the invention. The effect of the chemical on a second messenger pathway of the cells is then measured using conventional techniques.

Cells and cell lines expressing mammalian gb2 $GABA_B$ receptors can be used to provide a sufficient amount of the proteins for the preparation of antibodies using conventional techniques. The antibodies can be used to define more clearly the localization of receptor types than is possible using in situ hybridization to mRNA. They could also be used for diagnostic purposes if clinically significant abnormalities were found in association with the gb2 $GABA_B$ receptors. Moreover, they would permit purification of the receptor proteins by affinity chromatography. Highly purified gb2 $GABA_B$ would permit the 3-dimensional protein to be studied by X-ray crystallography. Such information would be especially useful in rational drug design.

The molecular biology techniques used in making and using the present invention are generally known in the art. For example, suitable techniques and procedures are generally described in "Molecular Cloning: A Laboratory Manual," 2nd Ed., Sambrook, Fritsch, and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; and "Current Protocols in Molecular Biology," Ausubel et al., John Wiley and Sons, N.Y., 1987 (updated quarterly).

Hgb2 and rgb2 Amino Acid Sequences. Through EST data base searches, polymerase chain reaction, and library screening as described in the Examples, human and rat homologues of a novel receptor (hgb2 in human and rgb2 in rat) have been identified and isolated. These receptors have homology to the $GABA_B$ receptors (rgb1a and rgb1b from rat) identified by Kaupmann et al. (Nature 386, 239-246 (1997)). Figure 1 shows a schematic of the hgb2, rgb2, rgb1a and rgb1b receptor nucleotide sequences denoting the placement of oligonucleotide primers and restriction enzyme sites used to generate each of these constructs and the expression plasmids. Hgb2 receptor fragments were amplified from human brain cDNA while rgb2 was isolated from a rat cortex cDNA library followed by amplification of rat brain cDNA to obtain the 5' end of the receptor including the start site. The resulting 5786 base pair sequence generated for hgb2 includes an open reading frame of 2826 bases. The combined 5' RACE (i.e., rapid amplification of cDNA ends) product and insert for rgb2 spans 5459 base pairs and includes an open reading frame of 2823

bases. The cDNA sequences for hgb2 and rgb2 predict proteins of 942 and 941 amino acid. respectively. with relative molecular mass of 104 kD.

Sequence similarity and hydropathy profiles (Kyte et al., J. Mol. Biol. 157. 105-132 (1982) of the predicted proteins demonstrate that they share structural features with the $GABA_B$ receptor rgb1 (Kaupmann et al., Nature 386. 239-246 (1997)). the parathyroid cell calcium-sensing receptor, and the family of metabotropic glutamate receptors (for review see Pin and Duvoisin, Neuropharmacol. 34, 1-26 (1995)). Significant features in the hgb2 and rgb2 sequences include 7 putative transmembrane regions, a large extracellular amino terminus of 476 residues. and 4 potential sites for N-linked glycosylation (Figure 2). There is an abundance of proline residues in the amino terminus of both hgb2 and rgb2, with a string of 9 proline residues only 9 residues into the sequences.

Comparison of the amino acid sequences for hgb2 and rgb2 with that for rgb1a and rgb1b (Figure 2) shows 28% amino acid identity which rises to 38% when conservative amino acid substitutions are taken into account. Homology between the gb2 and gb1 receptors is not limited to the hydrophobic regions but instead spans the entire protein. Search of Genbank indicated that hgb2 and rgb2 share significant sequence homology with the metabotropic glutamate receptors. The Genbank search also showed a limited degree of homology between the amino acid sequence of hgb2 and rgb2 and the human and rat calcium-sensing receptors.

Hgb2 Encodes a $GABA_B$ Receptor. A functional screen of HEK293 cells stably transfected with pcD3.1hgb2 or pcD3.1rgb1a revealed several cell lines expressing these receptors to varying degrees. Preliminary data is shown in Figure 3 for the stable cell lines hgb2/293-10 and rgb1a/293-18. Adenylyl cyclase was stimulated as described in the Example 8 with 10 uM forskolin in the presence or absence of 30 uM baclofen or 30 uM GABA. 100 uM AOAA (a GABA transaminase inhibitor), and 100 uM nipecotic acid (a GABA uptake blocker). hgb2/293-10 cells exhibited a 65% reduction in forskolin-stimulated cAMP synthesis with baclofen and a 50% decrease with GABA plus AOAA and nipecotic acid (Figure 3). A 40% reduction in forskolin-stimulated cAMP synthesis was observed for rgb1a/293-18 cells treated with baclofen and a 45% decrease was observed for GABA plus AOAA and nipecotic acid (Figure 3). Neither baclofen nor GABA plus AOAA and nipecotic

acid in the absence of forskolin had any affect on cAMP synthesis (Figure 3). These data suggest that the hgb2 receptor, like the rgb1a and rgb1b receptors, is a $GABA_B$ receptor since the criteria for baclofen sensitivity has been established. Kaupmann et al. (Nature 386, 239-246 (1997)) observed similar reduction in forskolin-stimulated cAMP synthesis with HEK293 cells stably expressing the rgb1a receptor. However, it is worth noting that it took 300 uM baclofen to observe a 30% decrease in forskolin-stimulated cAMP synthesis as compared to the 30 uM used in the current study which accomplished a 40% reduction. These differences may be due to differences in the levels of receptor expression between the rgb1a stable cells and rgb1a/293-18 cells. Acquisition of a radiolabeled antagonist for binding analyses will be necessary before differences in receptor cell number may be addressed. Work is underway to further characterize the pharmacology of these receptors and to establish a rank order of potency for $GABA_B$ receptor agonists and antagonists.

Rgb2 Expression in Rat CNS and Peripheral Tissues. Analysis of Northern blots of human mRNA from several brain regions and peripheral tissues with a probe directed against the hgb2 receptor reveals a single mRNA species of 6.2 kb (Figure 4). Hgb2 receptor mRNA is expressed in all of the brain areas tested although the level of expression seems to vary significantly with the highest being found in the cortex and thalamus and the lowest in the corpus callosum, caudate nucleus, and medulla. In addition, a weak 6.2 kb band is detected in spinal cord mRNA. No hgb2 receptor mRNA is detected in any of the peripheral tissues examined with the exception of the heart where a weak band is observed.

In situ hybridization histochemistry was carried out using sense and antisense probes directed against both the rgb2 and rgb1 receptors for comparison as described in the Examples. The rgb1 receptor probe is directed against a region common to both the rgb1a and rgb1b receptors (see Examples and Figure 1) therefore mRNA for both receptors will be hybridized. Rgb1 receptor mRNA is heterogeneously expressed in abundance throughout all regions of the rat brain as previously described (Kaupmann et al., Nature 386, 239-246 (1997)). Rgb2 receptor mRNA is also abundant in rat brain, but its distribution is somewhat more restricted. There are significantly lower levels of rgb2 receptor mRNA hybridization in the caudate-putamen, septum, medial

basal hypothalamus, and brainstem of the rat brain as compared with levels of rgb1 receptor mRNA (Figures 5 and 6). The levels and distribution of rgb2 and rgb1 receptor mRNA hybridization in all other brain regions examined appeared to be nearly identical. In contrast to rgb1 receptor mRNA, hybridization to rgb2 receptor mRNA was not detected in white matter, suggesting that expression of rgb2 receptor mRNA is restricted to neurons. Kaupmann et al. (Nature 386, 239-246 (1997)) showed that rgb1 mRNA is expressed in neurons but not glia in the CA3 field of the hippocampus. Detailed examination of other brain regions has revealed that rgb1 mRNA is found in glia as well. While hybridization to rgb2 mRNA has not been detected in glia, more detailed studies of rgb2 mRNA expression and protein localization at the cellular level are necessary before this possibility can be ruled out.

While it is difficult to make a direct extrapolation from mRNA hybridization to protein location, the data presented herein are generally in agreement with previously published binding data (Chu et al., Neuroscience 34, 341-357 (1990); Bowery et al., Neuroscience 20, 365-383 (1987)). Binding of GABA in certain regions of the rat brain including the septal area, caudate putamen, hypothalamus and brainstem, with the exception of the interpeduncular nucleus, is relatively low (Chu et al., Neuroscience 34, 341-357 (1990); Bowery et al., Neuroscience 20, 365-383 (1987)) in agreement with the levels of hybridization which were detected for rgb2.

The following examples are intended to illustrate the invention and not to limit the invention. All references cited in the present specification are hereby incorporated by reference.

Example 1 -- Cloning of hgb2. A tblastn search of the Genbank EST database using the amino acid sequence of the rat gb1 receptor as query detected three ESTs (R76139, R80448, and Z43654) which showed distant homology to transmembrane domains 5-7 and another EST (T07261) which showed similarity to transmembrane domains 1-3. Primers designed from these sequences (JC136 gcgggatccCTTCGGCACGAATACCAGGCAGAGGGT for 5' extension; JC137 ccggaattcCAGAAGCTCATAAAGATGTCGAGTCC and JC135 ccggaattcTTCTTAGCTTTGGGAGACCCGCAACGT for 3' extension) were used

with the Clontech AP1 primer to do 5' and 3' RACE using Clontech Marathon-Ready human brain cDNA as template and touchdown PCR conditions (94°C. 30 sec and 72°C. 4 min for 5 cycles: 94°C. 30 sec and 70°C. 4 min for 5 cycles: 94°C. 30 sec and 68°C. 4 min for 20 cycles). JC135 and JC136 primers were derived from the transmembrane domains 5-7 ESTs while JC137 was derived from T07621. JC136 and JC137 primers were used together to generate a product of 722 bases which, when sequenced, confirmed that the ESTs were derived from the same mRNA. 3' products from RACE with JC135 and JC137 were reamplified with the Clontech AP2 primer yielding two products of 1.2 and 1.7 kb is size but lacking a poly(A) signal.

A tblastn search of the Genbank EST database with the 3' sequences detected an EST (AA323988) which overlapped with the query sequence and extended the sequence through an A-rich region. Gb2-15 (AGCACTAGAACTCCAGCTGGAAGTCA) was designed based on EST AA323988 and used in a 3' RACE reaction as described above to yield a 2.3 kb band that when sequenced was shown to include a poly(A) signal. 5' RACE with JC136 yielded a 0.88 kb Not I/BamH I fragment which when sequenced showed weak amino acid homology to rgb1. JC153 (GCCTACGATGGCATCTGGGTCATCGC) was designed based on the JC136 5' RACE product sequence and paired with JC136 to yield a 1.1 kb fragment. Further 5' extensions were obtained by using Clontech Advantage-GC PCR (1 µl GC-melt per 50 µl reaction, PCR conditions: 94°C, 15 sec and 68°C. 3.5 min for 30 cycles) and nested primers JC152 (CAGATTCCAGCCTTGGAGGGACTCTGC) followed by JC186 (TGCATCGTAGAAGGCTTTCAACCCTTTTGC) and adaptor primers AP1 followed by AP2.

pcDhgb2I was generated by subcloning a Nae I/Xho I fragment from a JC153/JC165 (JC165: AATAAGGCTCGAGGTCAGGTGCCAA) 2.2 kb PCR product and an EcoR I/Nae I fragment from a JC171/JC154 (JC171: CAGCAGCCCGCCGCTCTCCATCAT; JC154: gcgcgtaatacgactcactatagggGTAGTTGAAGTCCTGGATCC G) 1 kb PCR product into EcoR I/XhoI digested pcDNA1/amp (Invitrogen). The expression construct pcDhgb2II was constructed by subcloning a Not I/Xmn I 5' RACE fragment obtained with AP2/JC186 into a Not I/Xmn I digested pcDhgb2I. PcD3.1hgb2 was made by

subcloning an EcoR I/Xba I fragment from pcDhgb2 into EcoR I/Xba I digested pcDNA3.1 (Invitrogen).

Example 2 -- Cloning of rgb2. Rgb2 was obtained by screening pools of clones from a rat cortex cDNA library using Southern blot analysis. DNA was prepared from each of 49 pools representing an average of 5×10^5 clones per pool and digested with Xho I to release the inserts from the vector DNA. 5 µg of Xho I digested pool DNA was run on a 0.8 % agarose gel and transferred to a nitrocellulose supported membrane (BA-S 85. Schleicher & Schuell) using a TurboBlotter (Schleicher & Schuell). Nitrocellulose membranes were hybridized with a ^{32}P-labeled random primed (Boerhinger Mannheim) hgb2 JC137/JC136 (JC137: ccggaattcCAGAAGCTCATAAAGATGTCGAGTCC; JC136: gcgggatccCTTCGGCACGAATACCAGGCAGAGGGT) PCR fragment in 3x Denhardt's and 3x SSC at 60°C. Hybridization was followed by two 15 min washes in 3x SSC at 60°C. Blots were imaged on the Fujix PhosphorImaging system (Fuji). Bacteria from two pools was each used to make 22 subpools. DNA was prepared from these subpools and analyzed as described above. Four positive subpools (10I, 10M, 37O, and 37Y) were analyzed further in subpools representing 3×10^3 clones per subpool. Nitrocellulose filter lifts were made of successive platings of subpools 10I7 and 37O9 and processed following standard protocols ("Molecular Cloning: A Laboratory Manual," 2nd Ed., Sambrook, Fritsch, and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; and "Current Protocols in Molecular Biology," Ausubel et al., John Wiley and Sons, N.Y., 1987 (updated quarterly)). Filters were hybridized as described above followed by two washes with 3x SSC at 60°C for 15 min and 1 wash in 3X SSC at 60°C for 1 hr. Filters were exposed to BioMax MR film (Kodak) for 16 hr.

Two positive overlapping clones were isolated from this screen, 10I75a and 37O94a. The 5200 kb clone 37O94a was sequenced and found to lack a start site. RACE was performed using the high GC conditions described above with nested primers JC190 (CGCTGGTGCAGGGATCATTGGAGAA) and JC191 (CATATTCTGGTCAAACTGGCCAAGG) paired with AP1 followed by AP2 using Clontech Marathon-Ready rat brain cDNA as template to yield the 5' end of the rgb2

receptor. A Xho I/Bgl II fragment from the 5' RACE of rgb2 and a Bgl II fragment from 37O94a were subcloned into Xho I/BamH I digested pBSSK II (-). The correct orientation of the Bgl II fragment was established using restriction enzyme mapping with XhoI and BamH I. PcD3.1rgb2 was made by subcloning a Kpn I/BamH I fragment of the pBSSK II (-) plasmid with a BamH I/Xba I fragment from 37O94a into Kpn I/Xba I digested pcDNA3.1.

Example 3 -- Cloning of rgb1b and rgb1a. Rgb1b was obtained by amplification of Clontech rat brain Marathon-Ready cDNA with JC140 (ccggaattcccaccATGGGCCCGGGGGGACCCTGTACC) and JC141 (CTAGTCTAGATCACTTGTAAAGCAAATGTACTCGACT). pBSrgb1b was made by ligation of an EcoR I/Xba I fragment of rgb1b into EcoR I/Xba I prepared pBSSKII(-). pcDrgb1b was constructed by subcloning an EcoR I/Not I fragment from pBSrgb1b into EcoR I/Not I prepared pcDNA1/amp (Invitrogen). Rgb1a 5' sequence was obtained by 5' RACE with AP1 or 2 paired with JC144 (GAGTAG TTCGTACAAGTACTTGGTGGC) using Clontech rat brain Marathon-Ready cDNA as template. An rgb1a 5' fragment for vector construction was generated by amplification of the JC144 RACE product with JC139 (ccggaattcccaccATGCTGCTGCTGCTGCTGGTGCCTCTC) and JC144. pBSrgb1a was made by ligation of an EcoR I/Hpa I fragment from the JC139/JC144 PCR product into EcoR I/Hpa I digested pBSrgb1b. pcDrgb1a was constructed by subcloning an EcoR I/BstE II fragment from the pBSrgb1a into EcoR I/BstE II digested pcDrgb1b. An EcoR I/Not I fragment from pcDrgb1a was subcloned into pcDNA3.1 (Invitrogen) to make pcD3.1rgb1a.

Example 4 -- Northern Analysis. Clontech multiple human tissue Northerns were hybridized with the 0.88 kb Not I/BamH I fragment (bases 732-1613 of hgb2) at 42°C in 5X SSPE, 10X Denhardt's, 50% formamide, 2% SDS, 100 µg/ml denatured salmon sperm DNA. Following hybridization blots were washed three times for 10 min each in 0.2X SSPE, 1% SDS at 60°C. Blots were imaged on the Fujix PhosphorImaging system (Fuji).

Example 5 -- In Situ Hybridization Histochemistry. Preparation of rat brain sections, prehybridization and hybridization of rat brain slices was performed using conventional techniques (see, for example, Bradley et al., J. Neurosci. 12, 2288-2302 (1992); a general protocol can also be found on the following website: http://intramural.nimh.nih.gov/lcmr/snge/Protocol.html). Sections were hybridized with labeled antisense and sense riboprobes directed against rgb2 or rgb1. Probes were generated by amplification of rgb2 with JC216 paired with JC217 or with JC218 paired with JC219 (JC216: cgcgcaattaaccctcactaaaggACAACAGCAAACGTTCAGGC; JC217: gcgcgtaatacgactcactatagggCATGCCTATGATGGTGAG; JC218: cgcgcaattaaccctcactaaaggCTGAGGACAAACCCTGACGC; JC219: gcgcgtaatacgactcactatagggGATGTCTTCTATGGGGTC) or by amplification of rgb1 with JC160 paired with JC161 (JC160: cgcgcaattaaccctcactaaaggAAGCTTATCCACCACGAC; JC161: gcgcgtaatacgactcactatagggAGCTGGATCCGAGAAGAA) and labeled as described previously (Bradley et al., J. Neurosci. 12, 2288-2302 (1992); the general protocol can also be found at http://intramural.nimh.nih.gov/lcmr/snge/Protocol.html).

Example 6 -- Cell Culture and Stable Expression of hgb2 and rgb1a. HEK-293 cells, human embryonic kidney cells, were cultured in DMEM supplemented with 10% fetal calf serum (Sigma), 1% penicillin/streptomycin (GIBCO/BRL), at 37°C in 5% CO_2. HEK-293 cells grown to 75% confluency in 10-cm tissue culture plates were used for control, pcD3.1hgb2 and pcD3.1rgb1a transfections. HEK-293 cells were exposed to either a solution of cationic lipid/DNA (LipofectAMINE; GIBCO/BRL) in DMEM without fetal calf serum or penicillin/streptomycin (70 µg LipofectAMINE/10 µg of pcD3.1hgb2 or pcD3.1rgb1a) or a solution containing cationic lipid alone (70 µg LipofectAMINE) in DMEM, for 5 hr at 37°C. After incubation, lipid/DNA solutions were supplemented with equal volumes of DMEM with 20% fetal calf serum and cells were set at 37°C overnight. Within 24 hr of transfection media was removed and replaced with serum- and penicillin/streptomycin-supplemented DMEM. Transfected cells were allowed to recover for 24 hr after which each 10-cm plate was split to five 10-cm plates in

DMEM supplemented with 10% fetal calf serum, 1% penicillin/streptomycin and 375 μg/ml geneticin (G418; and GIBCO/BRL) for a period of 2-4 weeks. Resistant colonies were isolated and transferred to 24-well plates. Those cells that survived the isolation procedure were cultured for preparation of total RNA for dot blot analysis (Example 7) and for cAMP response assays (Example 8) to screen for expression of hgb2 and rgb1a.

Example 7 -- Detection of hgb2 and rgb1a RNA by Dot Blot Analysis. The cells from Example 6 were grown in 6-well plates to 75-100% confluency. Total RNA was prepared using the protocol provided with Trizol (GIBCO/BRL). Briefly, Trizol was added to each well and cells were triturated and transferred to 1.5 ml microfuge tubes. Samples were extracted with chloroform followed by precipitation with 2-propanol. Pellets were resuspended in 7.4% formaldehyde/6X SSC and stored at -20°C until analysis. Half of each sample was diluted in equal volume of 7.4% formaldehyde/6X SSC, denatured and applied to a supported nitrocellulose membrane using a dot blot apparatus. RNA was fixed to the membrane by UV crosslinking (Stratalinker, Stratagene) and blots were prehybridized in 6X SSC, 5X Denhardt's, 0.5% SDS, 50% formamide and 100 μg/ml denatured salmon sperm DNA at 42°C for at least 2 hr. Blots were hybridized with ^{32}P-labeled random primed PCR fragments for hgb2 and rgb1a (hgb2: JC137/JC136; rgb1a: JC160/JC161), 1-5 x 10^6 cpm/ml, were denatured and added directly to the prehybridized solution. Blots were hybridized at 42°C overnight followed by one 20 min wash at 25°C with 2X SSC, 0.1% SDS and two 30 min washes at 68°C with 0.2X SSC, 0.1% SDS. Blots were imaged on the Fujix PhosporImaging system. Data tabulation is underway.

Example 8 -- Determination of Cellular cAMP Response. HEK-293 cells from Example 6 which stably express the hgb2 and rgb1a receptors were lifted in 1X PBS, 2.5 mM EDTA, counted, pelleted and resuspended at 1.5 x 10^5 cells per 100 μl in Krebs-Ringer-Hepes medium, 100 μM Ro 20-1724 (RBI; Natick, MA) and incubated at 37°C for 20 min. 100 μl cells was added to 100 μl of prewarmed (37°C, 10 min) Krebs-Ringer-Hepes medium, 100 μM Ro 20-1724 without or with agonists, antagonists and/or forskolin. Following a 20 min incubation at 37°C the assay was

terminated by setting the cells on ice and centrifuging at 2000 rpm for 5 min at 4°C. 175 µl of assay solution was removed and replaced with 175 µl of 0.1 N hydrochloric acid, 0.1 mM calcium chloride and cells were set on ice for 30 min and then stored at -20°C. cAMP determinations were made using a solid phase modification (Maidment et al., Neurosci. 33, 549-557 (1989)) of the cAMP radioimmunoassay described by Brooker et al., Adv. Cyclic Nucleotide Res. 10, 1-33 (1979). Immulon II removawells (Dynatech; Chantilly, VA) were coated overnight with 100 µl of protein G (1 mg/ml in 0.1M $NaHC0_3$, pH 9.0) at 4°C. Prior to use protein G-coated plates were rinsed with PBS-gelatin-Tween (phosphate buffered saline containing 0.1% gelatin, 0.2% Tween-20) 3 times quickly and then once for 30 minutes. Following the rinse with PBS-gelatin-Tween the RIA was set up by adding 100 µl 50 mM sodium acetate, pH 4.75, cAMP standards or aliquots from treated cells, 5,000-7,000 cpm ^{125}I-succinyl cAMP, and 25 µl of a sheep antibody to cAMP diluted in 50 mM sodium acetate, pH 4.75 (Atto instruments; dilution of stock to $2.5x10^{-6}$, determined empirically) to the plates in a final volume of 175 µl. Plates were incubated 2 hr at 37°C or overnight at 4°C, rinsed 3 times with sodium acetate buffer, blotted dry, and then individual wells broken off and bound radioactivity determined in a gamma counter. EC_{50} values were determined by nonlinear least squares fit of the data to the equation

$$Y = A_o + (A_m * L)/(EC_{50} + L)$$

where Y is the amount of cCMP (picomole/well), A_o is the basal level of cAMP, A_m is the maximal accumulation under these conditions, L is the added ligand concentration, and EC_{50} the apparent half-maximal concentration for stimulation of cAMP accumulation. Data tabulation is underway.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Clark, J. and Bonner, T.

(ii) TITLE OF INVENTION: Mammalian gb2 $GABA_B$ Receptors

(iii) NUMBER OF SEQUENCES: 4

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: Windows

(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

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(C) CLASSIFICATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5786 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AAATAACAAG ACCCACAGAA GTGACTCTAG CTACTTAATG GTTCTGTTCT TTTATATGCA   5580
GCAAACACAC CGTCCATTTC TGAAGAGGCT TCGGCCTGAA GGCATTTTCC AATGATGTTA   5640
GTGCACAAAA CGCTTTAAAT TAGACTGGAA CTGCCAGAAT CAAATGTAAA TGAGGAATTT   5700
CTCGTACCCC TACTGCATGG TATCGATTTT TAATAAATTG TTGCAAATTT GTTTTTATGA   5760
ATAAAAGGAA AAAACCTGTC GTCTTT                                        5786
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5459 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
GGCCGTTCTG AGCGAGCAGA AGCCGGAGCC GAGCCGGGGC CCGTGCCGGC GCCATTGCGC     60
GGGRGCCGCG GGCAAAKCTC GGCGCCGGGC GGCGGGCCGG GCCAGGCCAT GCGGGCCGAG    120
TGAGCTGGCG CCCGCAGCCC GCGGCGCGGC ATGGCTTCCC CGCCGAGCTC CGGGCAGCCC    180
CGGCGGGCGC GGCGGCGCGC GGGGCCCGCG CGCGTGCTGC TGCGGCTGCT GCTGTCGGTG    240
CTGCTGTGGT TGGCGCCCGG GGCCTGGGGC TGGACGCGGG GCGCCCCCCG GCCGCCGCCC    300
AGCAGCCCGC CGCTCTCCAT CATGGGCCTC ATGCCGCTCA CCAAGGAGGT GGCCAAGGGC    360
AGCATCGGGC GCGGCGTGCT CCCCGCCGTG GAGCTAGCCA TCGAGCAGAT CCGCAACGAG    420
TCACTCCTGC GCCCCTACTT CCTGGACCTG CGACTCTATG ACACCGAGTG TGACAATGCA    480
AAGGGACTGA AAGCCTTCTA TGACGCAATA AAGTATGGGC CGAACCATTT GATGGTGTTT    540
GGAGGCGTCT GTCCGTCTGT CACATCTATT ATCGCGGAGT CCCTCCAAGG CTGGAATCTG    600
GTGCAGCTTT CCTTCGCCGC CACCACGCCT GTTCTTGCGG ATAAGAAGAA GTACCCGTAT    660
TTCTTCCGGA CGGTGCCGTC AGACAACGCG GTGAACCCCG CCATCCTGAA GCTCCTGAAG    720
CACTTCCGCT GGCGGCGTGT GGGCACACTC ACGCAGGACG YGCAGCGCTT CTCCGAGGTG    780
AGGAATGACC TGACTGGGGT TCTGTATGGG GAAGATATTG AGATCTCAGA CACAGAGAGT    840
TTCTCCAATG ATCCCTGCAC CAGCGTCAAA AAGCTCAAGG GGAATGACGT GCGGATCATC    900
CTTGGCCAGT TTGACCAGAA TATGGCAGCA AAAGTCTTCT GTTGTGCCTT CGAGGAGAGC    960
ATGTTTGGCA GCAAGTACCA GTGGATCATC CCGGGATGGT ACGAGCCTGC GTGGTGGGAG   1020
CAGGTGCATG TGGAGGCCAA TTCCTCACGC TGCCTGCGCA GAAGCCTCCT GGCTGCCATG   1080
GAAGGTTACA TCGGAGTGGA CTTTGAGCCC CTGAGCTCCA AACAAATCAA GACCATCTCA   1140
GGGAAGACTC CACAGCAGTT TGAAAGAGAG TACAACAGCA AACGTTCAGG CGTGGGGCCC   1200
AGCAAGTTCC ATGGGTACGC CTACGATGGG ATCTGGGTCA TCGCCAAGAC CCTACAGAGG   1260
GCCATGGAGA CACTGCATGC CAGTAGCAGG CACCAGCGGA TCCAGGACTT CAACTACACA   1320
GACCACACGC TGGGCAAAAT CATCCTCAAT GCCATGAACG AGACCAACTT CTTCGGGGTC   1380
ACGGGTCAAG TTGTGTTCCG GAACGGGGAG AGAATGGGAA CCATTAAATT TACTCAATTT   1440
CAAGACAGCA GAGAGGTGAA GGTCGGCGAA TACAACGCGG TGGCTGACAC ACTGGAGATC   1500
ATCAATGACA CCATAAGGTT CCAGGGGTCC GAGCCACCCA AGGACAAGAC CATCATTCTG   1560
GAGCAGCTTC GGAAGATCTC GCTTCCACTG TATAGCATCC TGTCCGCTCT CACCATCCTC   1620
GGCATGATCA TGGCCAGCGC CTTCCTCTTC TTCAACATCA AGAACCGGAA CCAAAAGCTG   1680
ATTAAGATGT CAAGCCCCTA CATGAACAAC CTCATCATCC TGGGAGGAAT GCTGTCCTAT   1740
GCATCCATCT TCCTCTTTGG CCTCGACGGG TCCTTCGTCT CAGAAAAGAC CTTTGAAACA   1800
CTCTGCACGG TCCGGACCTG GATTCTCACC GTGGGCTACA CAACTGCCTT TGGGGCCATG   1860
TTTGCAAAGA CCTGGAGGGT CCATGCCATC TTCAAAAATG TGAAGATGAA GAAGAAGATC   1920
ATCAAAGACC AGAAGCTGCT TGTGATTGTG GGGGGCATGC TGCTCATCGA CCTGTGCATC   1980
CTGATCTGTT GGCAGGCTGT GGACCCCCTG CGGAGGACAG TGGAGAGGTA CAGCATGGAG   2040
CCGGACCCAG CAGGCCGGGA CATCTCCATC CGCCCATTGC TGGAACACTG CGAAAACACC   2100
CACATGACCA TCTGGCTTGG CATTGTCTAC GCCTACAAGG GGCTCCTCAT GCTATTCGGT   2160
TGTTTCTTGG CATGGGAAAC CCGCAATGTG AGCATCCCTG CCCTCAACGA CAGCAAGTAC   2220
ATCGGCATGA GTGTGTACAA TGTGGGGATC ATGTGCATCA TCGGGGCTGC TGTCTCCTTC   2280
CTGACGCGTG ACCAGCCCAA CGTGCAGTTC TGCATCGTGG CCCTGGTCAT CATCTTCTGC   2340
AGCACCATCA CTCTCTGCCT GGTGTTTGTG CCAAAGCTCA TCACTCTGAG GACAAACCCT   2400
GACGCAGCCA CTCAGAACAG GCGGTTCCAG TTCACACAGA ACCAGAAGAA AGAAGATTCG   2460
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AAGACCTCCA GTTCAGTCAC CAGCGTGAAC CAGGCGAGCA CGTCACGCCT GGAGGGACTG   2520
CAGTCAGAAA ACCACCGCCT TCGAATGAAG ATCACAGAGC TGGACAAAGA CTTGGAAGAA   2580
GTCACCATGC AGCTACAAGA CACACCAGAG AAGACCACAT ACATCAAAGA GAATCACTAC   2640
CAAGAGCTCA ACGACATCCT CAGCTTGGGC AACTTCACAG AGAGCACAGA TGGAGGAAAG   2700
GCCATTCTAA AAAATCACCT CGATCAAAAC CCCCAGCTCC AGTGGAACAC GACAGAGCCC   2760
TCAAGAACAT GCAAAGACCC CATAGAAGAC ATCAACTCCC CGGAGCACAT CCAGCGCCGG   2820
CTGTCGCTCC AGCTCCCCAT CCTTCACCAC GCCTACCTCC CATCCATCGG AGGCGTGGAT   2880
GCCAGCTGCG TCAGCCCCTG TGTCAGCCCT ACCGCCAGCC CTCGCCACAG ACACGTACCA   2940
CCCTCCTTCC GAGTCATGGT CTCGGGCCTG TAGGGGTGGG AGGCCTGGGG CCGGGGCCTC   3000
CCCGGGACAG CACCATGCTG GGCCAAGGCG CCTGCCACAG GCACACTGAC GGCGGCGAGA   3060
AGCTGGGCAC CATGCTGCCT CTCCAGACTG CTGGAATGGC GCTCAGGCAG AGCGGGACTC   3120
GGCACCGACC TCGAGCCTTA TCTGTGAAGG TCTTACTCTC ACAGAGGAGA GGAATGACAA   3180
TGACTTCTCC TTCTTGGCGT CTGCAAACAA AGAGGAGTTG GGATGTCTGA AACTTGCAAW   3240
AACAAATCAA ACTCTAGACA AAGGAGAGAG GCCTCGGACT CCTGCTGTCC TCGCCAAGTG   3300
GCCAGAGCAA GGGCTCTGCA GAAATGGCCC TCGGCACTCT GCACACACGT CATCGGTGGT   3360
GCCCGCCCCC ACACACCAGC CCTTCTCCAC TCTGGGGGAG AAGGTGAGCA GGGCCACCAG   3420
GTCCCCAGCA GCTGGCCCAG TCCACTTCAG ACTTCCTGGA ACAAGGGAGT CAGCAGGAAG   3480
GGTAGCCGGG CTCTGCCCTG GGGCTGGGGG AATCAGAGCA AGCAGCTGCG CATGCTCCCA   3540
GGGGACATCC ATCAGAAGAG AGATGTTTGT TACTCTCTGA TAAATGCCAC ACATTAACAC   3600
AATAACACCC GTTCCGGGGA CTGTGGGGAT TCAGGGCACC GCACTGCAGA CATGCTCTGC   3660
AGGATGCAAC AGACAGTCTG TCATGCACCT ACCGTGTCGG CCATGTTCTT GTGTTCAAAT   3720
TTAGGGTTCC CGGTAATAAG GGGAACCATC TGAGCTAATC ATGGAATGTC TGCTCCCAGA   3780
AAACACGATA AAGAAAGATT GTGCACTTTA ACCTCTCCCA TGGGGGCCCA AGGGCTGCTG   3840
GGATTCCCCC TCTCCCAACA CACTGACTTT GTTTTTGACC AAAGTGAATC AGGGGCATTC   3900
TGCTAAAAGT CATCTCTGTG TTCCTGAGCT AGAGCTGCTG GCCCAGGGCC CCCTGGCTTC   3960
CAGAAGGTAG CCCTCCAGTG CCCCTCCAGC CCTAACCCGT GAGTGCCCAC CTAGGGTGTC   4020
ACCTGAGGCG GTCARTCAGC AGGCTGGTTC CCATCCATCA CGACATACTT TCCTTGTAAT   4080
TGAACCCCAA AGAGCACTGG CCTGGGAGTC AGACCTGGAT TTGAGTCCTC AGTCCCTTCA   4140
GACTCTCTCC GTGACCTTGG GCAAGTCACC AGTGTCTCTC TGAGCCTCGG TTTCCCCTCT   4200
GTAACAGACT GGGGTTGAAA TAACACCCAT CCTGCCTACC TCACAGGGTC ACTCTGAGGA   4260
TGGACACTTG ATCTCATTCA TCCAGGAGAG CTTTGCACTG GGAAGCAGCT CTGACCTGTG   4320
AGGTATTAGT ACGACCATGA CCTTCAGCCC TCCCACCAGC TGGGGAGGGT GCGGTGCGGC   4380
CCAGAGAAAC TTCCTGCAAG GCCCTCTACC TAGGGACAGG AACAGCCCAG TCAGAGGCAG   4440
GCTCAGGACT CTTCTGACAT TCCTTAATAG GTTCATTCTT TGCTAAAGGC CTGAATGGCA   4500
CCRTCCAGGG GCGTCTGCAC AAACGAGTGG TTTGCTCACT CCCTAAAGAA TCCTAAAGGC   4560
AGCTTCAACA GGCAGGCAAT AATCTCTTCC CAGACCACTG CAGTCAGGAA CACACTGCTG   4620
TCAACCACCA GGCTGTGACG AAAGGGCCCA CGGGAATCAC CATCGCCAAC ATTTCAAAAC   4680
ACCCTAGTTC ACGTAGCATA GCTCTTCCTC CCCATCCCTC AAAGAGAGGT TACAGAGGTT   4740
CTGTAGCTTT TAAGGGGGGA AAAATGTCAA CACATCACAG GTCAAGTTGA TGTCCTTCTC   4800
TGTTTAGGCA CCAAAACTCC GTGTTGTCAC TCACTGTGTG TGTTAACCAG TATTTCCTTG   4860
CTTTTTCGAC TTCACCAAAA CCAAATTTCA TTTAAAAGAC CGYATTAGTT TTTTTCAAAG   4920
GGAAAGAGAT AATTAACTGT ACATAATGTA TACAGACAAA ACAAAACCTG TAGAAATATT   4980
ACTCCAGCAT AGCAGGAAAA AAAAAAAAAC AAAAAACAAA AAACAAAAGT ATTGGATTGT   5040
CGGAGGTGAG CGTGCGTCTG TAATCCCTTG TGACTCCGAA CTGTGCTCCG TCTTCTAGGT   5100
TAACCCACAA GATACATCTC TGTCTACTGA TCCTGTAGGT TCACCCATTT TTTTTTAATT   5160
TCTCTGCAAT TAACAAGCCC CACAAAGTGA TTCTGGCTAT CTACTGGTTC TGTTCTTTTA   5220
TATGCAGCAA ACACACGGTC CATTTCCTAG AGGCTTCCGC CCAAGGGCAT CTTCCAGTGA   5280
CGCTAGTGCG CACACACACA CACACACACA CACACACACA CACACACACA CACACACGAC   5340
TGCTTTCAAT TAGACCGGAA CTGGCAGAAT CAAATGTAAA TGAGGAGTTT CTCTCGCCCC   5400
ACTGCATGGT ATCGATTTTT AATAAATTGT TGCAAATTTG TTTTTATGAA TAACAGGGG     5459
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 940 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ala Ser Pro Arg Ser Ser Gly Gln Pro Gly Pro Pro Pro Pro Pro
 1               5                  10                  15
Pro Pro Pro Pro Ala Arg Leu Leu Leu Leu Leu Leu Leu Pro Leu Leu
            20                  25                  30
Leu Pro Leu Ala Pro Gly Ala Trp Gly Trp Ala Arg Gly Ala Pro Arg
        35                  40                  45
Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu
    50                  55                  60
Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala
65                  70                  75                  80
Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro
                85                  90                  95
Tyr Phe Leu Asp Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
            100                 105                 110
Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
        115                 120                 125
Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
    130                 135                 140
Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro
145                 150                 155                 160
Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
                165                 170                 175
Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Tyr
            180                 185                 190
Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe Ser
        195                 200                 205
Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu
    210                 215                 220
Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val Lys
225                 230                 235                 240
Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln
                245                 250                 255
Asn Met Ala Ala Lys Val Phe Cys Cys Ala Tyr Glu Glu Asn Met Tyr
            260                 265                 270
Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ser Trp
        275                 280                 285
Trp Glu Gln Val His Thr Glu Ala Asn Ser Ser Arg Cys Leu Arg Lys
    290                 295                 300
Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu Pro
305                 310                 315                 320
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Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln Gln
                325                 330                 335
Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser Gly Val Gly Pro Ser Lys
            340                 345                 350
Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr Leu
        355                 360                 365
Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile
    370                 375                 380
Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Arg Ile Ile Leu Asn
385                 390                 395                 400
Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val Phe
                405                 410                 415
Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln Asp
            420                 425                 430
Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu
        435                 440                 445
Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys
    450                 455                 460
Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu
465                 470                 475                 480
Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala Ser
                485                 490                 495
Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile Lys
            500                 505                 510
Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met Leu
        515                 520                 525
Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser
    530                 535                 540
Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr
545                 550                 555                 560
Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg
                565                 570                 575
Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys
            580                 585                 590
Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu
        595                 600                 605
Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val
    610                 615                 620
Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile
625                 630                 635                 640
Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp Leu
                645                 650                 655
Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys Phe
            660                 665                 670
Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser
        675                 680                 685
Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile
    690                 695                 700
Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe
705                 710                 715                 720
Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys
                725                 730                 735
Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala
            740                 745                 750
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Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu
        755                 760                 765
Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr
    770                 775                 780
Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys
785                 790                 795                 800
Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln
                805                 810                 815
Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu
            820                 825                 830
Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly
        835                 840                 845
Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln
    850                 855                 860
Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp
865                 870                 875                 880
Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro
                885                 890                 895
Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser
            900                 905                 910
Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg His
        915                 920                 925
Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu
    930                 935                 940
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 940 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Ser Pro Pro Ser Ser Gly Gln Pro Arg Pro Pro Pro Pro Pro
 1               5                  10                  15
Pro Pro Pro Ala Arg Leu Leu Leu Pro Leu Leu Leu Ser Leu Leu Leu
            20                  25                  30
Trp Leu Ala Pro Gly Ala Trp Gly Trp Thr Arg Gly Ala Pro Arg Pro
        35                  40                  45
Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu Thr
    50                  55                  60
Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala Val
65                  70                  75                  80
Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro Tyr
                85                  90                  95
Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
            100                 105                 110
Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
        115                 120                 125
Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
    130                 135                 140
Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro
145                 150                 155                 160
Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
                165                 170                 175
Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Phe
            180                 185                 190
Arg Trp Arg Arg Val Gly Thr Leu Thr Gln Asp Xaa Gln Arg Phe Ser
        195                 200                 205
Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu
    210                 215                 220
Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val Lys
225                 230                 235                 240
Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln
                245                 250                 255
Asn Met Ala Ala Lys Val Phe Cys Cys Ala Phe Glu Glu Ser Met Phe
            260                 265                 270
Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ala Trp
        275                 280                 285
Trp Glu Gln Val His Val Glu Ala Asn Ser Ser Arg Cys Leu Arg Arg
    290                 295                 300
Ser Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu Pro
305                 310                 315                 320
Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln Gln
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                325                 330                 335
Phe Glu Arg Glu Tyr Asn Ser Lys Arg Ser Gly Val Gly Pro Ser Lys
            340                 345                 350
Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr Leu
        355                 360                 365
Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile
    370                 375                 380
Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Lys Ile Ile Leu Asn
385                 390                 395                 400
Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val Phe
                405                 410                 415
Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln Asp
            420                 425                 430
Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu
        435                 440                 445
Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys
    450                 455                 460
Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu
465                 470                 475                 480
Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala Ser
                485                 490                 495
Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile Lys
            500                 505                 510
Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met Leu
        515                 520                 525
Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser
    530                 535                 540
Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr
545                 550                 555                 560
Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg
                565                 570                 575
Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys
            580                 585                 590
Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu
        595                 600                 605
Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val
    610                 615                 620
Glu Arg Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile
625                 630                 635                 640
Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp Leu
                645                 650                 655
Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys Phe
            660                 665                 670
Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser
        675                 680                 685
Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile
    690                 695                 700
Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe
705                 710                 715                 720
Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys
                725                 730                 735
Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala
            740                 745                 750
Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu
```

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        755                 760                 765
Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr
    770                 775                 780
Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys
785                 790                 795                 800
Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln
                805                 810                 815
Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu
            820                 825                 830
Leu Asn Asp Ile Leu Ser Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly
        835                 840                 845
Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln
    850                 855                 860
Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp
865                 870                 875                 880
Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro
                885                 890                 895
Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser
            900                 905                 910
Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg His
        915                 920                 925
Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu
    930                 935                 940
```

CLAIMS

We claim:

1. An isolated and essentially pure DNA molecule which encodes a mammalian gb2 $GABA_B$ receptor.

2. The isolated and essentially pure DNA molecule of claim 1, wherein the DNA molecule is a cDNA molecule.

3. The isolated and essentially pure DNA molecule of claim 2, wherein the mammalian gb2 $GABA_B$ receptor is a human gb2 $GABA_B$ receptor.

4. The isolated and essentially pure DNA molecule of claim 2, wherein the cDNA molecule has the sequence given by SEQ. ID NO.:1 or allelic variations thereof.

5. The isolated and essentially pure DNA molecule of claim 2, wherein the mammalian gb2 $GABA_B$ receptor is a rat gb2 $GABA_B$ receptor.

6. The isolated and essentially pure DNA molecule of claim 2, wherein the cDNA molecule has the sequence given by SEQ. ID NO.:2 or allelic variations thereof.

7. An isolated mammalian $GABA_B$ receptor of subtype gb2.

8. The isolated mammalian $GABA_B$ receptor of claim 7, wherein the mammalian gb2 $GABA_B$ receptor is a human gb2 $GABA_B$ receptor.

9. The isolated mammalian $GABA_B$ receptor of claim 7, wherein the human gb2 $GABA_B$ receptor has the sequence given by SEQ. ID NO.:3 or allelic variations thereof.

10. The isolated mammalian $GABA_B$ receptor of claim 7, wherein the mammalian gb2 $GABA_B$ receptor is a rat gb2 $GABA_B$ receptor.

11. The isolated mammalian $GABA_B$ receptor of claim 7, wherein the rat gb2 $GABA_B$ receptor has the sequence given by SEQ. ID NO.:4 or allelic variations thereof.

12. A recombinant DNA comprising a DNA molecule which encodes a mammalian gb2 $GABA_B$ receptor and which is operably linked to control nucleotide sequences capable of expressing the mammalian gb2 $GABA_B$ receptor in a compatible host.

13. The recombinant DNA of claim 12, wherein the mammalian gb2 $GABA_B$ receptor is a human mammalian gb2 $GABA_B$ receptor.

14. An isolated or pure clone from a mammalian tissue DNA library, wherein the clone contains DNA which encodes a mammalian gb2 $GABA_B$ receptor.

15. The isolated or pure clone of claim 14, wherein the clone is from a human tissue library and the DNA encodes a human gb2 $GABA_B$ receptor.

16. A sequencing vector for sequencing DNA which encodes a mammalian gb2 $GABA_B$ receptor, said vector comprising a sequencing vector and DNA, which encodes a mammalian gb2 $GABA_B$ receptor, inserted into the vector in proper orientation and correct reading frame for sequencing the DNA.

17. The sequencing vector of claim 16, wherein the DNA encodes a human gb2 $GABA_B$ receptor.

18. An expression vector for expressing DNA that codes for a mammalian gb2 $GABA_B$ receptor in a compatible host, said expression vector comprising a vector capable of transforming a procaryotic or eucaryotic cell and DNA which encodes a mammalian gb2 $GABA_B$ receptor inserted into the vector in proper orientation and correct reading frame for expression.

19. The expression vector of claim 18, wherein the DNA encodes a human gb2 $GABA_B$ receptor.

20. The expression vector of claim 19 wherein the cell is a mammalian cell.

21. A process for producing a mammalian gb2 $GABA_B$ receptor, said method comprising expressing the expression vector of claim 18 in the cell transformed by the vector.

22. A cell transformed with the expression vector of claim 18.

23. A mammalian cell line comprising a mammalian cell stably transfected with the expression vector of claim 18 and capable of continuous growth in a suitable culture medium.

24. The mammalian cell line of claim 23 wherein the mammalian cell is selected from the group consisting of a neuronal cell, a fibroblast cell, an epithelial-like cell, and a glial cell.

25. The mammalian cell line of claim 23 wherein the cells of the cell line express a human gb2 $GABA_B$ receptor having the amino acid sequence of SEQ. ID NO.:3 and allelic variants thereof.

26. A process for producing a human gb2 $GABA_B$ receptor, said process comprising culturing the mammalian cell line of claim 25 in a suitable culture medium.

27. A method of making a mammalian cell that expresses a mammalian gb2 $GABA_B$ receptor, said method comprising the steps of:

transfecting a mammalian cell that does not express the receptor with the expression vector of claim 182; and

recovering said transfected mammalian cell.

28. The method of claim 27, wherein the mammalian cell that does not express the receptor is taken from a cell line that is capable of continuous growth in a suitable culture medium and comprising the further step of cloning the transfected cell, thereby producing a cell line that is capable of continuous growth in a suitable culture medium, the cell line expressing mammalian gb2 $GABA_B$ receptor.

29. The method of claim 28, wherein the mammalian gb2 $GABA_B$ receptor is a human gb2 $GABA_B$ receptor.

30. The mammalian cell produced by the method of claim 29

31. The mammalian cell of claim 30, wherein the mammalian gb2 $GABA_B$ receptor is a human gb2 $GABA_B$ receptor.

32. In a method for the pharmacological, physiological, functional, or other investigational analysis of a mammalian gb2 $GABA_B$ agonist or antagonist, the improvement comprising the use of an effective amount of the mammalian cell of claim 30 in the pharmacological, physiological, functional, or other investigational analysis.

33. An *in vitro* method of determining the ability of a chemical to bind to a mammalian gb2 $GABA_B$ receptor, said method comprising the steps of:

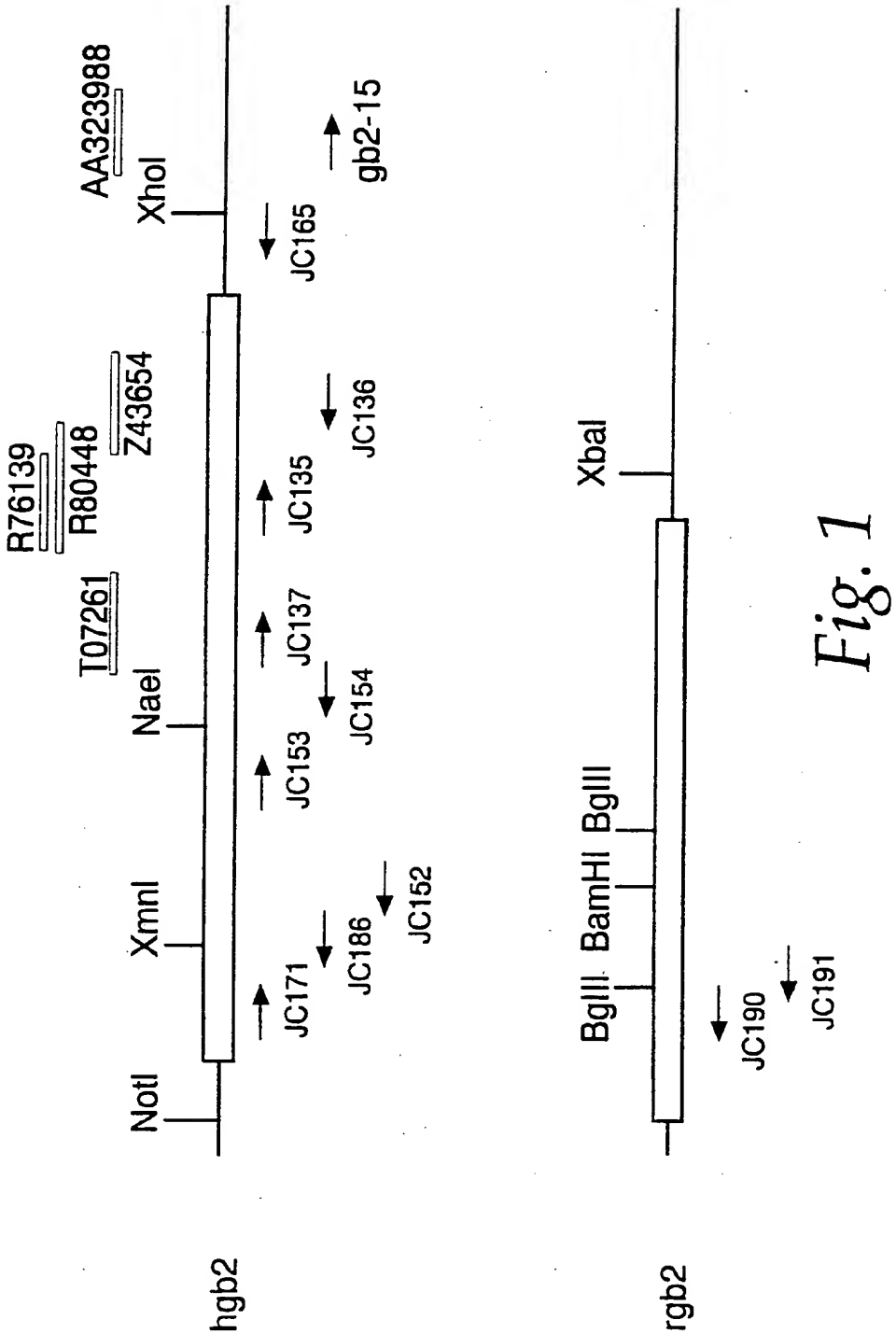
contacting the chemical with a cell or part thereof from the mammalian cell of claim 23; and

determining the ability of said cell or part thereof to bind the chemical.

34. An in vitro method for evaluating a chemical to determine if it is a mammalian gb2 $GABA_B$ receptor agonist. said method comprising the steps of:

contacting the chemical with a cell or part thereof from the mammalian cell line of claim 23; and

measuring the effect of the chemical on a second messenger pathway of the cell or part thereof.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JC190
JC191
rgb2

Fig. 1

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hgb2  --------------------------------------------------
rgb2  --------------------------------------------------
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rgb1b --------------------------------------------------

hgb2  --------------------------------------------------
rgb2  --------------------------------------------------
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rgb1b ----------------------------------------------S-

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rgb1b HS-PHLPRPHPRVPPHPSSERRAVYIGALFPMS-GGWPG--GQACQPAVEMAL
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          *
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rgb1  EDVNSRRDILPDYELKLIHHDSKCDPGQATKYLYELLYNDPIKIILMPG-CSS
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rgb2  VTSIIAESLQGWNLVQLSFAATTPVLADKKKYPYFFRTVPSDNAVNPAILKLL
rgb1  VSTLVAEAARMWNLIVLSYGSSSPALSNRQRFPTFFRTHPSATLHNPTRVKLF
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rgb2  KHFRWRRVGTLTQDCQRFSEVRNDLTGVLYGEDIEISDTESFSNDPCTSVKKL
rgb1  EKWGWKKIATIQQTTEVFTSTLDDLEERVKEAGIEITFRQSFFSDPAVPVKNL
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       *
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rgb1  DPSINCTVEEMTEAVEGHITTEIVMLNPANTRSISNMTSQEFVEKLTKRLKRH
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Fig. 2A

```
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       *                   TM1
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Fig. 2B

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hgb2(c)  NTTEPSRTCKDPIEDINSPEHIQRRLSLQLPILHHAYLPSIGGVDASCVS
rgb2     NTTEPSRTCKDPIEDINSPEHIQRRLSLQLPILHHAYLPSIGGVDASCVS
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hgb2(b)  ----------RHVPPSFRVMVSGL
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hgb2(c)  RGRGRLGT
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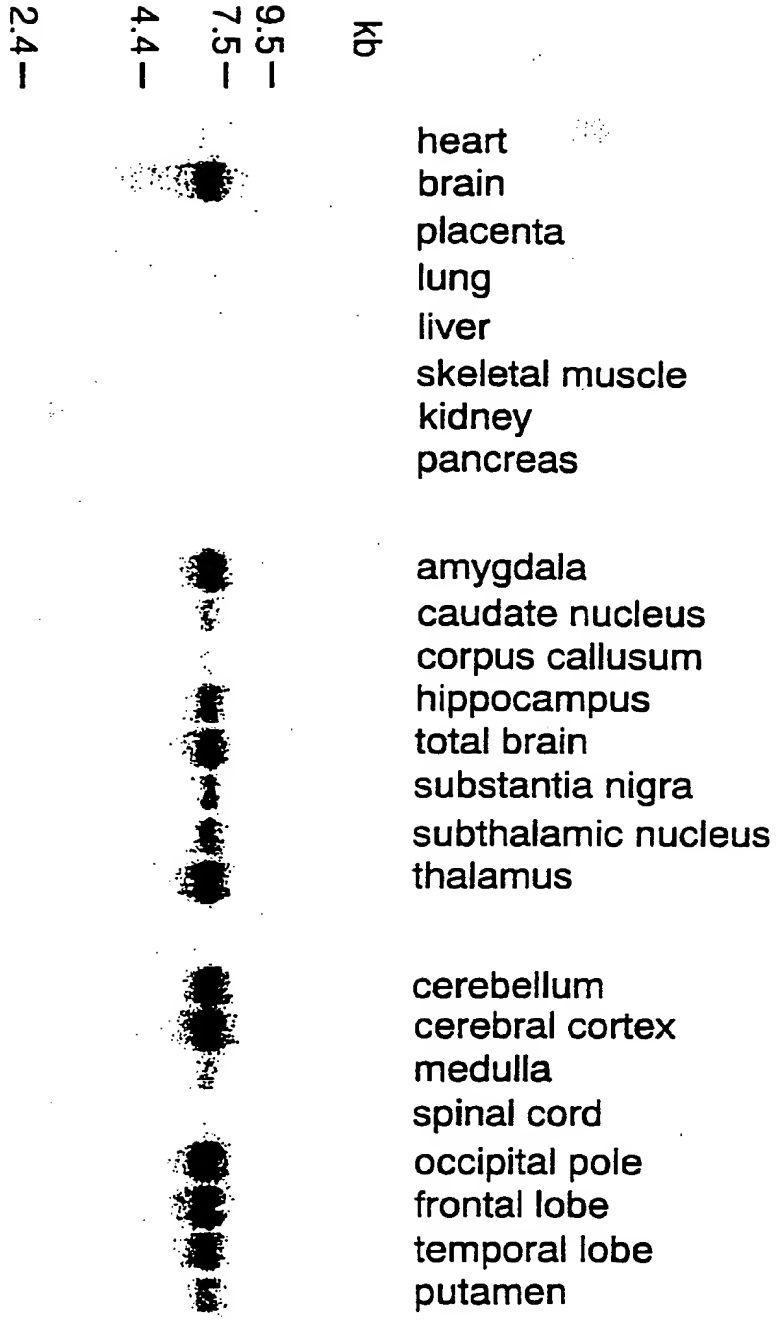
Fig. 2C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corpus callusum
hippocampus
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occipital pole
frontal lobe
temporal lobe
putamen

Fig. 3

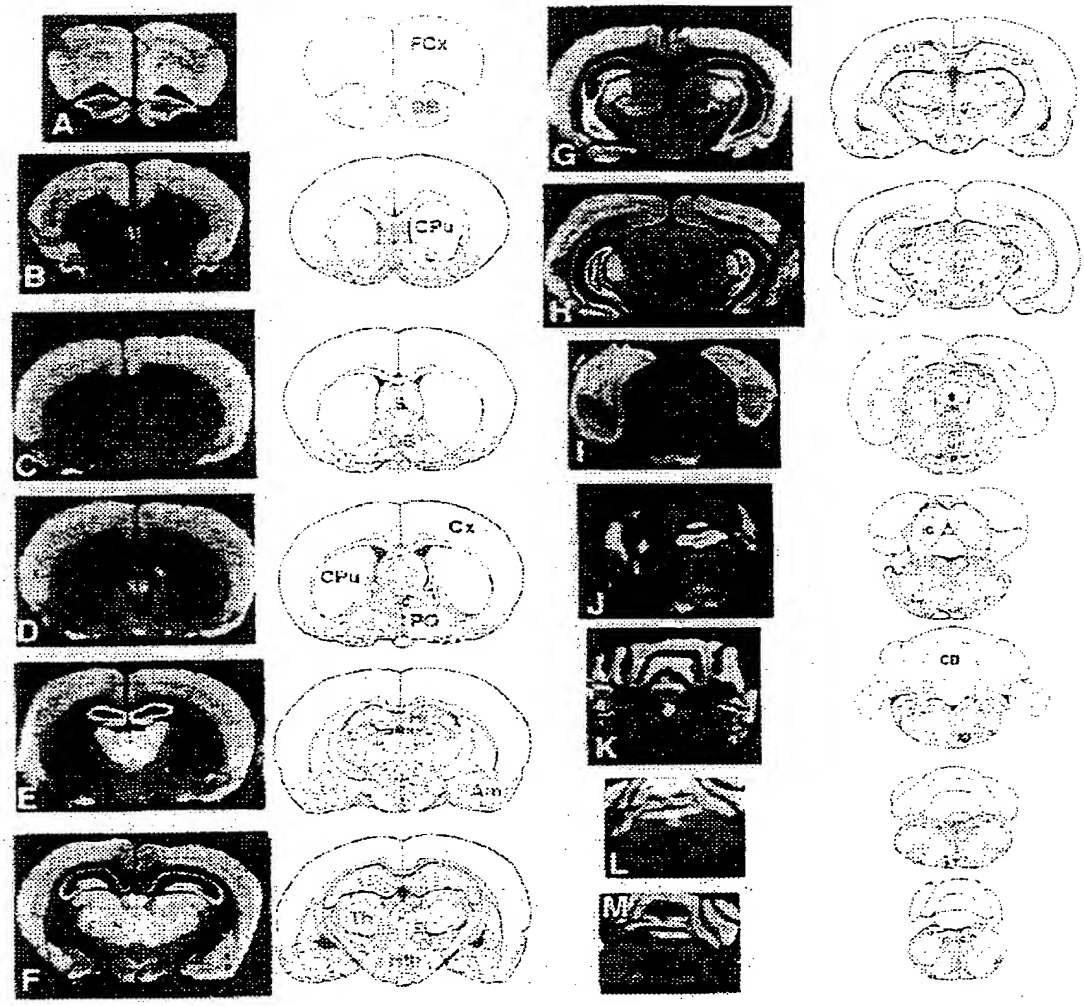

FIGURE 4

FIGURE 5

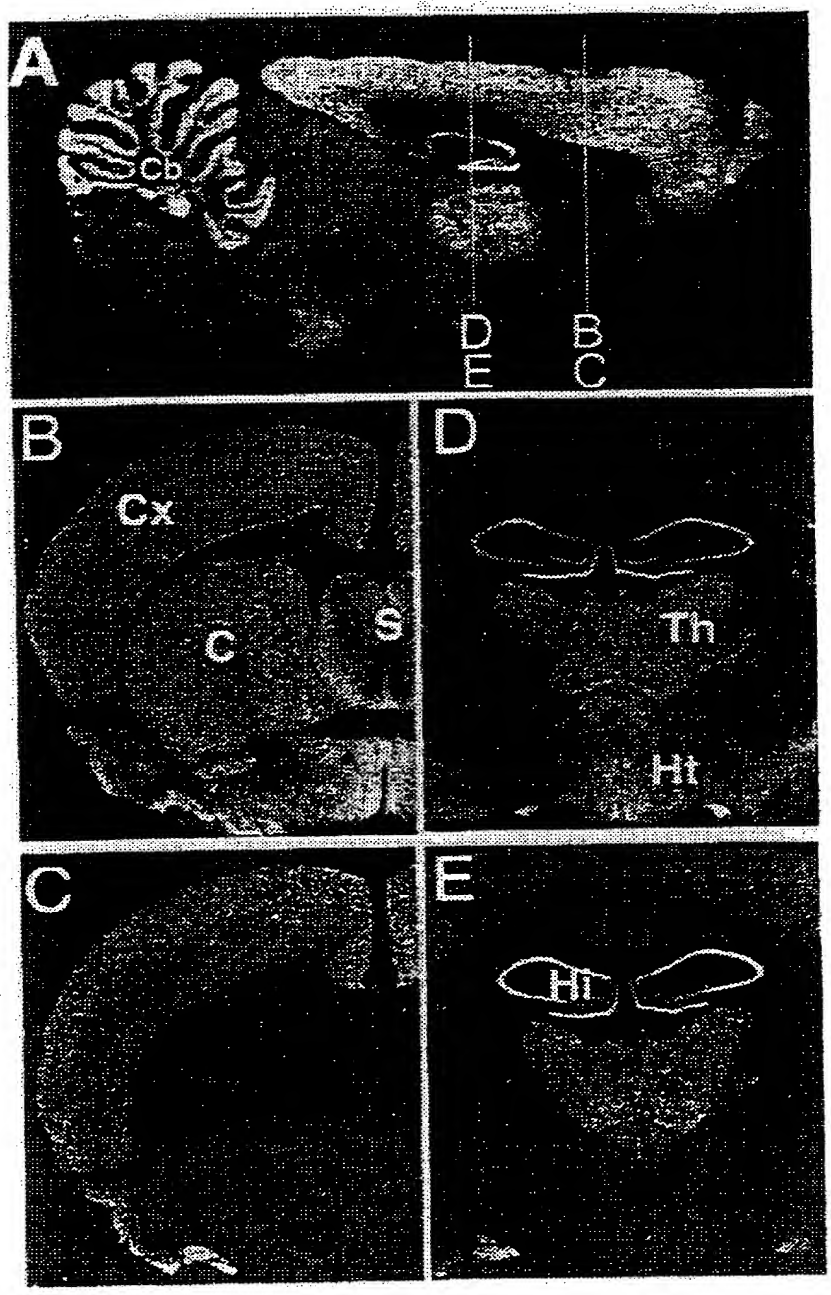

FIGURE 6

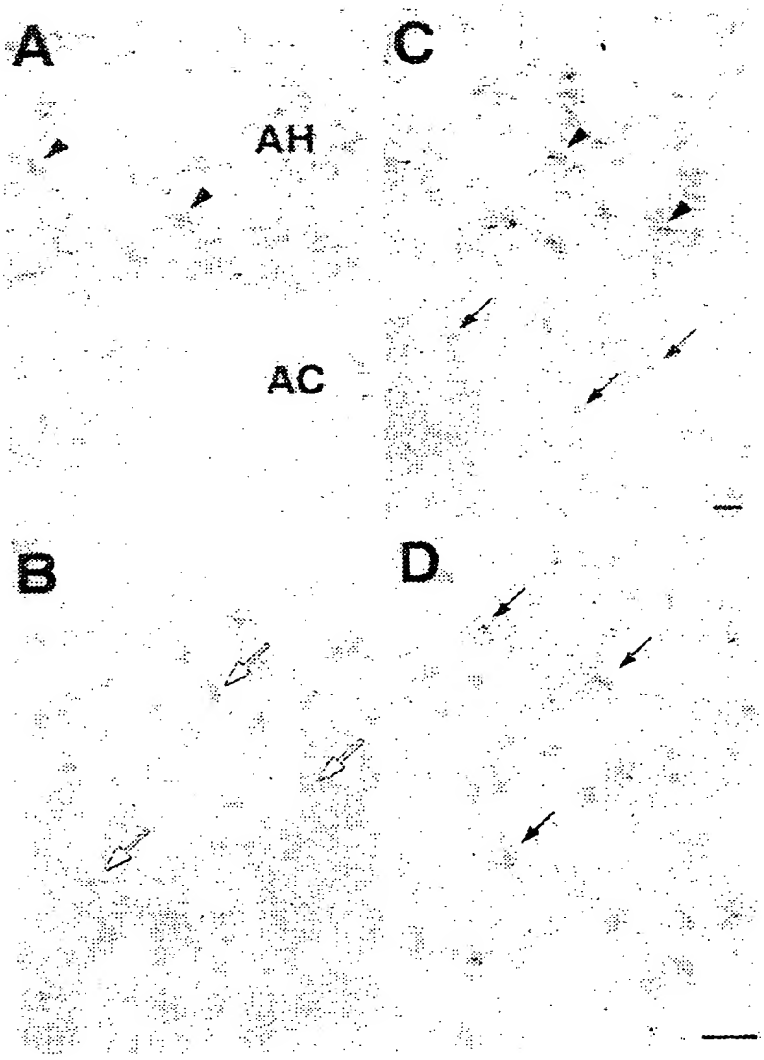

FIGURE 7

A

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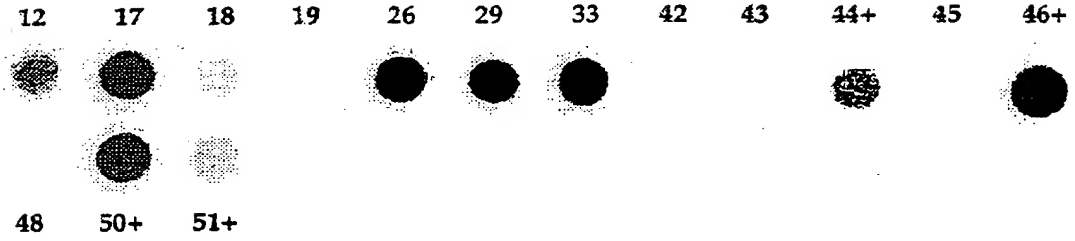
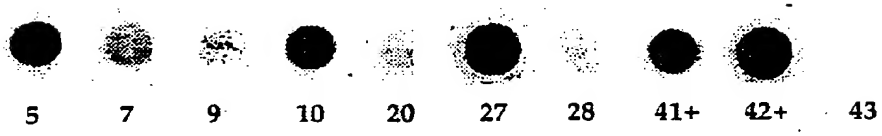


B.

HEK-293 hgb2 stable cell lines



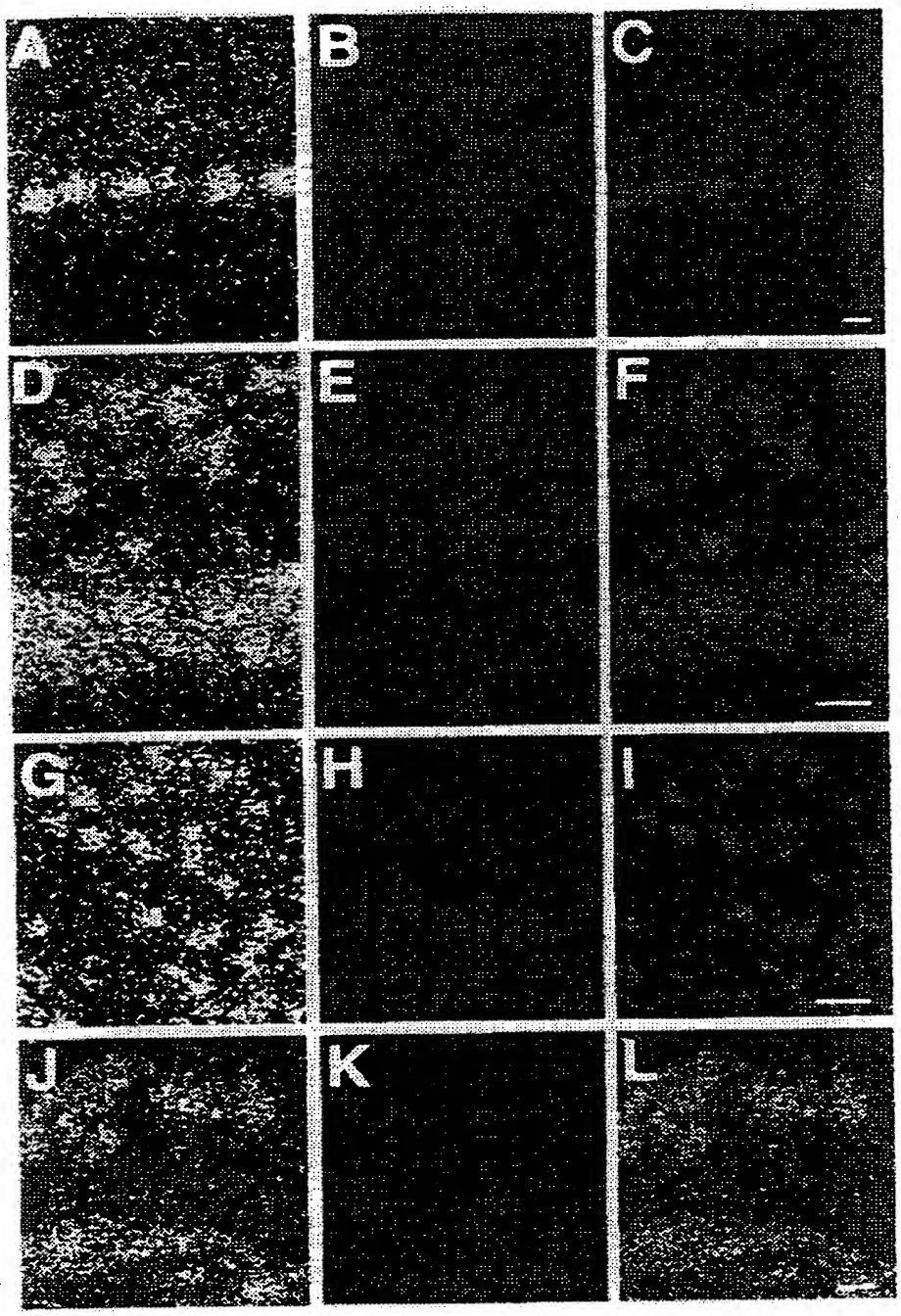
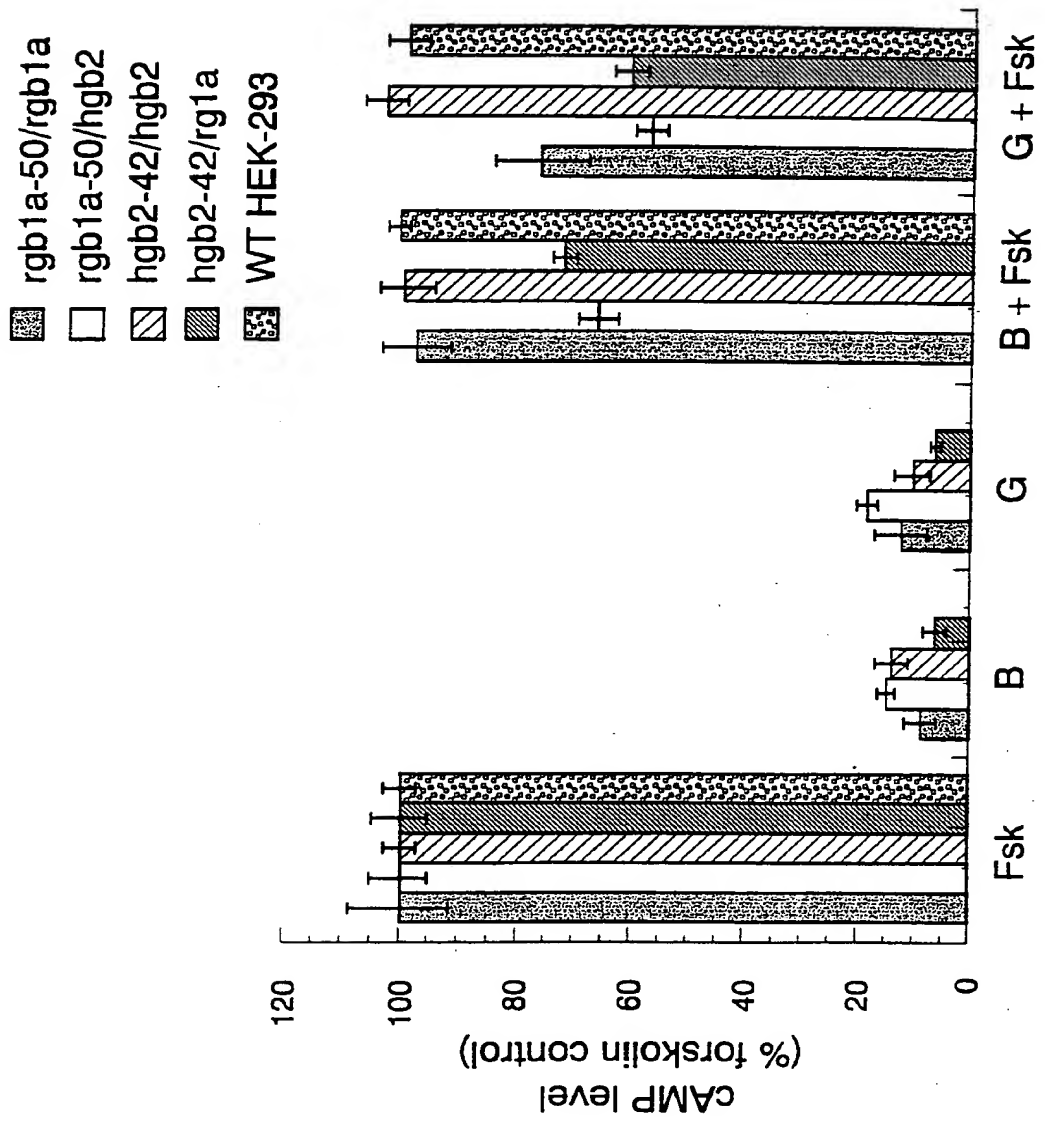


FIGURE 8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60
40
20
0
Fsk
B
G
B + Fsk
G + Fsk

Fig. 9

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<210> 2
<211> 5459
<212> DNA
<213> Rattus norvegicus

<400> 2

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cgctagtgcg cacacacaca cacacacaca cacacacaca cacacacaca cacacacgac    5340
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<210> 3
<211> 941
<212> PRT
<213> Homo sapiens

<400> 3

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Leu Pro Leu Ala Pro Gly Ala Trp Gly Trp Ala Arg Gly Ala Pro Arg
        35                  40                  45
Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu
    50                  55                  60
Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala
65                  70                  75                  80
Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro
                85                  90                  95
Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys
            100                 105                 110
Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu
        115                 120                 125
Met Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu
    130                 135                 140
Ser Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr
145                 150                 155                 160
Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val
                165                 170                 175
Pro Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His
            180                 185                 190
Tyr Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe
        195                 200                 205
Ser Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile
    210                 215                 220
Glu Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val
225                 230                 235                 240
Lys Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp
                245                 250                 255
Gln Asn Met Ala Ala Lys Val Phe Cys Cys Ala Tyr Glu Glu Asn Met
            260                 265                 270
Tyr Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ser
        275                 280                 285
Trp Trp Glu Gln Val His Thr Glu Ala Asn Ser Ser Arg Cys Leu Arg
    290                 295                 300
Lys Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu
305                 310                 315                 320
Pro Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln
                325                 330                 335
Gln Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser Gly Val Gly Pro Ser
            340                 345                 350
Lys Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr
        355                 360                 365
Leu Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg
    370                 375                 380
Ile Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Arg Ile Ile Leu
385                 390                 395                 400
Asn Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val
                405                 410                 415
Phe Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln
            420                 425                 430
Asp Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr
        435                 440                 445
Leu Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro
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    450                 455                 460
Lys Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro
465                 470                 475                 480
Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala
                485                 490                 495
Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile
            500                 505                 510
Lys Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met
        515                 520                 525
Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val
    530                 535                 540
Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu
545                 550                 555                 560
Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp
                565                 570                 575
Arg Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile
            580                 585                 590
Lys Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp
        595                 600                 605
Leu Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr
    610                 615                 620
Val Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser
625                 630                 635                 640
Ile Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp
                645                 650                 655
Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys
            660                 665                 670
Phe Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp
        675                 680                 685
Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile
    690                 695                 700
Ile Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln
705                 710                 715                 720
Phe Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu
                725                 730                 735
Cys Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp
            740                 745                 750
Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys
        755                 760                 765
Glu Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser
    770                 775                 780
Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met
785                 790                 795                 800
Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu
                805                 810                 815
Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln
            820                 825                 830
Glu Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe Thr Glu Ser Thr Asp
        835                 840                 845
Gly Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu
    850                 855                 860
Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu
865                 870                 875                 880
Asp Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu
                885                 890                 895
Pro Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala
            900                 905                 910
Ser Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg
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His Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu
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<210> 4
<211> 940
<212> PRT
<213> Rattus norvegicus

<400> 4

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Pro Pro Pro Ala Arg Leu Leu Leu Pro Leu Leu Leu Ser Leu Leu Leu
            20                  25                  30
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Trp Leu Ala Pro Gly Ala Trp Gly Trp Thr Arg Gly Ala Pro Arg Pro
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Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu Thr
    50                  55                  60
Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala Val
65                  70                  75                  80
Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro Tyr
                85                  90                  95
Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
            100                 105                 110
Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
        115                 120                 125
Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
    130                 135                 140
Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro
145                 150                 155                 160
Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
                165                 170                 175
Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Phe
            180                 185                 190
Arg Trp Arg Arg Val Gly Thr Leu Thr Gln Asp Xaa Gln Arg Phe Ser
        195                 200                 205
Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu
    210                 215                 220
Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val Lys
225                 230                 235                 240
Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln
                245                 250                 255
Asn Met Ala Ala Lys Val Phe Cys Cys Ala Phe Glu Glu Ser Met Phe
            260                 265                 270
Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ala Trp
        275                 280                 285
Trp Glu Gln Val His Val Glu Ala Asn Ser Ser Arg Cys Leu Arg Arg
    290                 295                 300
Ser Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu Pro
305                 310                 315                 320
Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln Gln
                325                 330                 335
Phe Glu Arg Glu Tyr Asn Ser Lys Arg Ser Gly Val Gly Pro Ser Lys
            340                 345                 350
Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr Leu
        355                 360                 365
Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile
    370                 375                 380
Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Lys Ile Ile Leu Asn
385                 390                 395                 400
Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val Phe
                405                 410                 415
Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln Asp
            420                 425                 430
Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu
        435                 440                 445
Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys.
    450                 455                 460
Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu
465                 470                 475                 480
Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala Ser
                485                 490                 495
Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile Lys
            500                 505                 510
Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met Leu
        515                 520                 525
Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser
    530                 535                 540
Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr
545                 550                 555                 560
Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg
                565                 570                 575
Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys
            580                 585                 590
Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu
        595                 600                 605
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Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val
    610                 615                 620
Glu Arg Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile
625                 630                 635                 640
Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp Leu
                645                 650                 655
Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys Phe
            660                 665                 670
Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser
        675                 680                 685
Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile
    690                 695                 700
Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe
705                 710                 715                 720
Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys
                725                 730                 735
Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala
            740                 745                 750
Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu
        755                 760                 765
Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr
    770                 775                 780
Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys
785                 790                 795                 800
Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln
                805                 810                 815
Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu
            820                 825                 830
Leu Asn Asp Ile Leu Ser Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly
        835                 840                 845
Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln
    850                 855                 860
Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp
865                 870                 875                 880
Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro
                885                 890                 895
Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser
            900                 905                 910
Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg His
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Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu
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<210> 6
<211> 35
<212> DNA
<213> Artificial Sequence

<400> 6
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<210> 7
<211> 36
<212> DNA
<213> Artificial Sequence

<400> 7
ccggaattct cttagcttt gggagacccg caacgt 36

<210> 8
<211> 26
<212> DNA
<213> Artificial Sequence

<400> 8
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<210> 9
<211> 26
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<213> Artificial Sequence

<400> 9
gcctacgatg gcatctgggt catcgc 26

<210> 10
<211> 27
<212> DNA
<213> Artificial Sequence

<400> 10
cagattccag ccttggaggg actctgc 27

<210> 11
<211> 30
<212> DNA
<213> Artificial Sequence

<400> 11
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<210> 12
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<211> 3396
<212> DNA
<213> Homo sapiens

<400> 28

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<211> 914
<212> PRT
<213> Homo sapiens

<400> 29

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Leu Pro Leu Ala Pro Gly Ala Trp Gly Trp Ala Arg Gly Ala Pro Arg
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Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu
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Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro
                85                  90                  95
Tyr Phe Leu Asp Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
            100                 105                 110
Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
        115                 120                 125
Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
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145                 150                 155                 160
Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
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Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Tyr
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Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe Ser
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Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln
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Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser Gly Val Gly Pro Ser Lys
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Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile
    370                 375                 380
Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Arg Ile Ile Leu Asn
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Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln Asp
            420                 425                 430
Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu
        435                 440                 445
Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys
    450                 455                 460
Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu
465                 470                 475                 480
Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala Ser
                485                 490                 495
Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile Lys
            500                 505                 510
Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met Leu
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Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser
    530                 535                 540
Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr
545                 550                 555                 560
Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg
                565                 570                 575
Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys
            580                 585                 590
Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu
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Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val
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Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile
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675 680 685
Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile
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705 710 715 720
Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys
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755 760 765
Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr
770 775 780
Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys
785 790 795 800
Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln
805 810 815
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820 825 830
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835 840 845
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850 855 860
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865 870 875 880
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<210> 30
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Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu
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65 70 75 80
Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro
85 90 95
Tyr Phe Leu Asp Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
100 105 110
Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
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Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
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145 150 155 160
Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
165 170 175
Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Tyr
180 185 190
Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe Ser
195 200 205
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Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln
                245                 250                 255
Asn Met Ala Ala Lys Val Phe Cys Cys Ala Tyr Glu Glu Asn Met Tyr
            260                 265                 270
Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ser Trp
        275                 280                 285
Trp Glu Gln Val His Thr Glu Ala Asn Ser Ser Arg Cys Leu Arg Lys
    290                 295                 300
Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu Pro
305                 310                 315                 320
Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln Gln
                325                 330                 335
Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser Gly Val Gly Pro Ser Lys
            340                 345                 350
Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr Leu
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Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile
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Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Arg Ile Ile Leu Asn
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Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val Phe
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Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln Asp
            420                 425                 430
Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu
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Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys
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Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu
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Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser
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Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr
545                 550                 555                 560
Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg
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Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu
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Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val
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Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile
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            660                 665                 670
Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser
        675                 680                 685
Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile
    690                 695                 700
Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe
705                 710                 715                 720
Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys
                725                 730                 735
Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala
            740                 745                 750
Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu
        755                 760                 765
Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr
    770                 775                 780
Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys
785                 790                 795                 800
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Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln
                805                 810                 815
Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu
            820                 825                 830
Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly
        835                 840                 845
Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln
    850                 855                 860
Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp
865                 870                 875                 880
Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro
                885                 890                 895
Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser
            900                 905                 910
Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg Thr
        915                 920                 925
Thr Leu Gly Arg Gly Val Cys Cys Arg Asn Thr Val Gly Ser Gly Cys
    930                 935                 940
Gly Glu Ala Gly His His Gly Trp Pro Leu Arg Thr Thr Arg Met Ala
945                 950                 955                 960
Leu Arg Trp Thr Gly
                965
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11869

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12N 15/10, 15/12, 5/10; C07K 14/705, 16/28; G01N 33/53
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.21, 69.1, 320.1, 325, 252.3, 254.11; 530/350, 387.9, 388.22; 536/23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, APS, GenBank
search terms: j. clark, t bonner, gabab, gb2, r2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	Database GenBank, CLARK et al. 'gb2, a second GABA-B receptor. Homo sapiens GABA-B receptor mRNA, complete cds.'. 08 October 1998, Accession Number AF056085.	1-4
X, P	Database GenBank, CLARK et al. 'gb2, A second GABA-B receptor. Rattus norvegicus GABA-B receptor gb2 mRNA, complete cds.'. 15 Oct ober 1998, Accession Number AF058795.	1, 2, 5
X, P	Database GenBank, CLARK et al. 'gb2, a second GABA-B receptor. Homo sapiens GABA-B receptor splice variant 1 mRNA, partial cds.' 08 October 1998, Accession Number AF095723.	1-4

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 SEPTEMBER 1999	Date of mailing of the international search report 18 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CLAIRE M. KAUFMAN Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11869

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	Database GenBank, CLARK et al. 'gb2, a second GABA-B receptor. Homo sapiens GABA-B receptor splice variant 2 mRNA, parital cds.'. 08 October 1998, Accession Number AF095724.	1-4
X, P	Database GenBank, LIU et al. 'Cloning and characterization of a novel human GABA-B receptor subtype with high affinity for GABA and low affinity for baclofen'. 17 May 1999, Accession Number AF095784.	1-3
X, P	Database GenBank, BOROWSKY et al. 'Direct Submission. Homo sapiens GABA-B receptor 2 mRNA, complete cds.' 07 January 1999, Accession Number AF074483.	1-3
X, P	WHITE et al. Heterodimerization is required for the formation of a functional GABA(B) receptor. Nature. 24 April 1999. Vol. 396, No. 6712, pages 679-682, especially Figure 1.	1-4, 6-11, 13-29
X, P	JONE et al. GABAB receptors function as a heterodimeric assembly of the subunits GABABR1 and GABABR2. Nature. 17 December 1998. Vol. 396, pages 674-678, especially Figure 1.	1,2,5,6, 9,12-14,17- 20, 23, 26, 29
X, P	KAUPMANN et al. GABAB-receptor subtypes assemble into functional heteromeric complexes. Nature. 17 December 1998. Vol. 396, pages 683-687.	1, 2, 5, 6, 9, 12-14, 17-20, 23, 26, 29, 30
X, P	KUNER et al. Role of heteromer formation in GABAB receptor function. Science. 01 January 1999. Vol. 283, pages 74-77.	1, 2, 5, 6, 9, 12-14, 17-20, 23, 26, 29

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11869

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.21, 69.1, 320.1, 325, 252.3, 254.11; 530/350, 387.9, 388.22; 536/23.1, 23.5

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